RSC Nanoscience & Nanotechnology

Edited by Qasim Chaudhry, Laurence Castle and Richard Watkins

Nanotechnologies in Food

RSC Publishing

Nanotechnologies in Food

RSC Nanoscience & Nanotechnology

Series Editor:
Professor Paul O'Brien, *University of Manchester, UK*
Professor Sir Harry Kroto FRS, *University of Sussex, UK*
Professor Harold Craighead, *Cornell University, USA*

Titles in the Series:
1: Nanotubes and Nanowires
2: Fullerenes: Principles and Applications
3: Nanocharacterisation
4: Atom Resolved Surface Reactions: Nanocatalysis
5: Biomimetic Nanoceramics in Clinical Use: From Materials to Applications
6: Nanofluidics: Nanoscience and Nanotechnology
7: Bionanodesign: Following Nature's Touch
8: Nano-Society: Pushing the Boundaries of Technology
9: Polymer-based Nanostructures: Medical Applications
10: Molecular Interactions in Nanometer Layers, Pores and Particles: New Findings at the Yoctovolume Level
11: Nanocasting: A Versatile Strategy for Creating Nanostructured Porous Materials
12: Titanate and Titania Nanotubes: Synthesis, Properties and Applications
13: Raman Spectroscopy, Fullerenes and Nanotechnology
14: Nanotechnologies in Food

How to obtain future titles on publication:
A standing order plan is available for this series. A standing order will bring delivery of each new volume immediately on publication.

For further information please contact:
Book Sales Department, Royal Society of Chemistry,
Thomas Graham House, Science Park, Milton Road, Cambridge,
CB4 0WF, UK
Telephone: +44 (0)1223 420066, Fax: +44 (0)1223 420247, Email: books@rsc.org
Visit our website at http://www.rsc.org/Shop/Books/

Nanotechnologies in Food

Edited by

Qasim Chaudhry, Laurence Castle and Richard Watkins
The Food and Environment Research Agency, Sand Hutton, York, UK

RSC Publishing

RSC Nanoscience & Nanotechnology No. 14

ISBN: 978 0 85404 169 5
ISSN: 1757 7136

A catalogue record for this book is available from the British Library

Published by The Royal Society of Chemistry,
Thomas Graham House, Science Park, Milton Road,
Cambridge CB4 0WF, UK

Registered Charity Number 207890

For further information see our web site at www.rsc.org

Preface

Rapid advancements in the fields of nanosciences and nanotechnologies in the past decade have not only led to a lot of hopeful anticipation, but have also raised some concerns. The current global market impact of nano-enabled products is in many billions of US$ and it is estimated by some to cross the 1 trillion US$ mark in a few years time. For such a rapidly expanding set of cross-cutting technologies, an obvious and prime target of new applications is the food sector, which itself is worth around 4 trillion US$ per annum globally. However, even at such an early stage, when the food and health food markets are only being 'tested' by market forces for new materials and products of nanotechnologies, they seem to have opened a new Pandora's box. There are mixed voices that are raising expectations and concern among the general public at the same time. Projections of enormous benefits are equally matched by calls for a moratorium or outright ban on the technologies until they are proven safe for human health and the environment. The same distinctive chemical and physical properties of nanomaterials that make them so attractive for new product development have raised fears over their safety to consumer health. A debate over how best to define nanomaterials, and whether they should be treated as new materials under the regulatory frameworks is still ongoing. Questions have also emerged over the adequacy and appropriateness of existing risk assessment paradigms, testing methodologies, detection and monitoring tools, as well as over the possible societal impacts of the new technologies.

Despite all this, it seems that many nano-sized materials have been a part of our everyday lives all the time, in the form of biological entities and processes that happen naturally at a nanoscale. Since the development of probe microscopes in the 1980s, food structures have been studied close to the molecular level. It is now known that most of our food materials are either composed of nanostructures, or are broken down into them during digestion. The concerns

RSC Nanoscience & Nanotechnology No. 14
Nanotechnologies in Food
Edited by Qasim Chaudhry, Laurence Castle and Richard Watkins

Published by the Royal Society of Chemistry, www.rsc.org

over deliberately added insoluble and bio-persistent nanoparticles in food do, however, seem justified. The prospect of being exposed through consumption of food and drinks to free, insoluble and possibly bio-persistent nanoparticles, which may have large reactive surfaces, and which may cross biological barriers to reach otherwise protected sites in the body is a legitimate worry. Such concerns, combined with the in-built scepticism of the general public towards any technologically derived food, have led to a call for more knowledge and understanding before such applications can be given what David Bennet has regarded in this book 'a license to produce' by the general public.

Against this contentious and rapidly changing background, this book puts the various views into perspective and analyses the pros and cons of the new technologies in an objective and realistic manner. The book presents the state-of-the-art in chapters written by leading experts in their respective fields. The subject areas cover science and technology, new product innovations, health and safety, consumer perception, risk assessment, risk management and regulatory aspects. The book aims to inform both non-specialist and specialist readers who are either new to the area or who want information and understanding from outside their immediate specialism. The Editors believe that this book, and of course the contributors to it, bring clarity to a number of issues and help move the debate on the new technologies forward in a more pragmatic manner.

Qasim Chaudhry
Laurence Castle
Richard Watkins

Contents

RSC Nanoscience & Nanotechnology No. 14
Nanotechnologies in Food
Edited by Qasim Chaudhry, Laurence Castle and Richard Watkins

Published by the Royal Society of Chemistry, www.rsc.org

CHAPTER 1

Nanotechnologies in the Food Arena: New Opportunities, New Questions, New Concerns

QASIM CHAUDHRY, RICHARD WATKINS AND LAURENCE CASTLE

The Food and Environment Research Agency, Sand Hutton, York YO41 1LZ, UK

1.1 Background

It has been suggested for sometime that materials and substances may be manipulated at the very small size scale through atom-by-atom assembly.[1] The advent of nanotechnology in recent years has provided a systematic way for the study and 'fine-tuning' of material properties in the nanometer size range. Nanotechnology is a broad term used to represent an assemblage of processes, materials and applications that span physical, chemical, biological and electronic science and engineering fields. The common theme amongst them is that they all involve manipulation of materials at a size range in the nanometer scale. One nanometer (nm) is one-billionth of a meter. A nanomaterial has been defined as a 'material having one or more external dimensions in the nanoscale or which is nanostructured',[2] where the nanoscale size range is approximately 1–100 nm (Figure 1.1). Materials with all three external dimensions in the nanoscale are classed as nanoparticles. Nanomaterials also exist in other forms, such as nanorods or nanotubes with two dimensions in the nanoscale, or nanolayers, coatings or sheets with just one dimension in the nanoscale.

RSC Nanoscience & Nanotechnology No. 14
Nanotechnologies in Food
Edited by Qasim Chaudhry, Laurence Castle and Richard Watkins

Published by the Royal Society of Chemistry, www.rsc.org

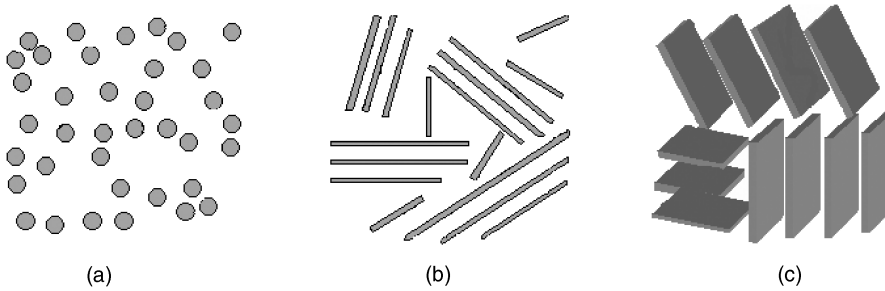

Figure 1.1 Nanomaterials as (a) particles; (b) rods; (c) layers.

Of particular interest to most nanotechnology applications are engineered nanoparticles (ENPs) that are manufactured specifically to achieve a certain material property or composition. Although ENPs are produced in free particulate forms, they tend to stick together to form larger agglomerates due to enormous surface free energies. In final applications, ENPs may be in fixed, bound or embedded forms in different matrices, such as food packaging plastics. Other applications, such as certain cosmetics, personal care products and functional foods may contain free ENPs. The chemical nature of substances used to manufacture ENPs can be inorganic (e.g. metals and metal oxides) or organic (e.g. food additives and cosmetics ingredients). Some nanomaterials are also obtainable from natural sources, most notably montmorillonite (also known as bentonite) that are nanoclays commonly obtained from volcanic ash/rocks. To help visualise nanomaterials in context, organic life is carbon based, and the C–C bond length is about 0.15 nm. So placed in a food context, most ENPs are bigger than molecules such as lipids, are a similar size to many proteins, but are smaller than the intact cells in plant- and animal-based foods (Figure 1.2).

The fundamental driver at the heart of most nanotechnology applications is the promise for improved or new functionalities of materials, and a possible reduction in the use of (chemical) substances. On an equivalent weight basis, ENPs have much larger surface to mass ratios (also known as the aspect ratio) due to their very small sizes compared to the conventional bulk forms. Thus, a relatively small amount of an ENP may provide a level of functionality that would otherwise require a much greater amount of the conventional material. The notion 'a little goes a long way' is probably the single most powerful reasoning behind many of the nanotechnology applications in different sectors. The very small size of ENPs can also offer other benefits. For example, nano-sizing of water-insoluble substances can enable their uniform dispersion in aqueous formulations. This makes it possible to reduce the use of solvents in certain applications such as cosmetics, paints and coatings, and allows the dispersion of food additives such as water-insoluble colours, flavours and preservatives in low-fat systems. Nano-sized nutrients and supplements have also been claimed to have a greater uptake, absorption and bioavailability in the body compared to bulk equivalents. This aspect alone has attracted a lot of

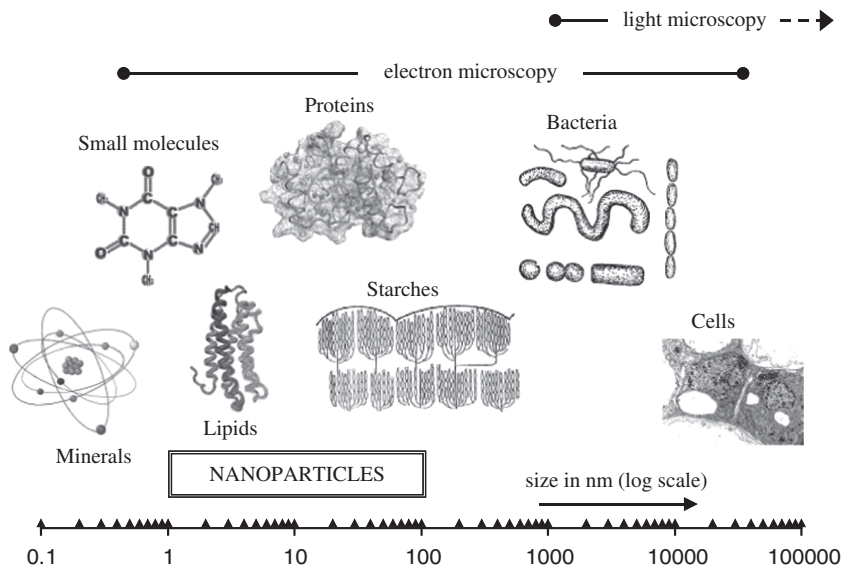

Figure 1.2 Nanomaterials placed in the context of other components in foods.

commercial interest in the use of nano-sized ingredients, supplements and nutraceuticals in (health)food applications.

The current applications of nanotechnology span a wide range of sectors, predominantly cosmetics and personal-care, health-care, paints and coatings and electronics. As in these sectors, nanotechnology is also promising to revolutionise the food industry – from food production, processing, packaging, transportation and storage to the development of new food tastes and textures and innovative food packaging applications. Nanotechnology has also emerged as one of the major converging technologies, offering the potential for further new developments through integration with other sciences and technological disciplines. Already there are examples where integration of nanotechnology with biotechnology and information technology is enabling the development of miniaturised devices, such as nanobiosensors. The use of the latter to detect pathogens and contaminants during food processing, transportation and storage is expected to enhance safety and security of food products. In view of the new technological developments, it is not surprising that the food industry is amongst the main sectors eagerly seeking ways to realise the potential benefits offered by nanotechnology.

This book is aimed at providing an impartial view of the potential prospects, benefits and risks that nanotechnology can bring to the food sector and its customers, and it also aims to discuss some of the main questions and concerns that the new technological developments have started to raise. In turn, this first chapter sets the scene for the subsequent chapters on individual application areas that are written by acknowledged experts in their respective fields.

1.2 Evolution of New Technologies in the Food Sector

The main driver that has shaped our present-day food industry is the basic human need for a sustained supply of safe, nutritious, affordable and enjoyable food throughout the year. Our food has gone through a long history of transformations over the centuries, from hunting and gathering to highly mechanised farming and technologically advanced processing and preservation methods. Agricultural food production during early human settlements is known to have started off with little knowledge, elementary tools and at the mercy of climate, pests and pathogens. The knowledge gained over generations enabled different civilisations to live off the land, and paved the way for systematic farming and animal breeding. The basic food production methods, however, then seem to have remained more or less unchanged over many centuries. By the early 1900s, agriculture was still run as a family-controlled or community-owned affair in most countries. The norms of food production, transportation and trade, however, started to transform in the 20th century with the introduction of mechanised farming, high-yielding crop varieties and, later on, with the availability of synthetic fertilisers, pesticides and other agrochemicals (antibiotics, hormones). The so-called 'green revolution' of the mid-20th century succeeded in substantially increasing the global food production. As the production of food reached industrial scales, new ways were found to transport, store and preserve foodstuffs. This laid the foundations of the modern-day food industry. The advancement in DNA technology in the past few decades has led to further advances in our understanding of the fundamental biological principles and genetic mechanisms, and enabled a big leap from protracted conventional breeding methods to faster knowledge-based improvements of crops and farm animals.

The history of food processing is also as old as that of food production. Throughout the centuries, foodstuffs have been processed and treated in various ways, and blended with different ingredients and additives to kill off pests and pathogens, to enhance nutritional value, taste, flavour and texture, and to keep and store foodstuffs for longer periods. In that respect, many of the processes used by the modern-day food industry, e.g. heat-treatment, fermentation, acid-hydrolysis, kilning, curing, smoking, drying etc, are not new to the consumer. However, the current consumer-driven food industry has to constantly look for innovative and novel products that not only offer new tastes, textures and flavours but are also wholesome, nutritious and value for money. The food sector now has a multitude of sub-sectors and branches that span from farm to fork. The global food retail market alone has been estimated to be worth between 3 and 4 trillion US$.[3] With globalisation of trade and industry worldwide, the rigid national boundaries that once existed in relation to food production and consumption have also become gradually obscure, and the supply and demand are now largely determined by global market forces. In this context, the introduction of nanotechnology is likely to make new waves in the already very competitive and technologically advanced food industry. These aspects are discussed in more detail in Chapters 2 and 7.

1.3 Public Perception of Nanotechnology Food Products

Before being successfully established, any new technology has to cross a number of technological, societal and regulatory barriers. This is especially true when the technology relates to such a sensitive area as food. The new nanotechnology-derived materials and applications for the food sector are not likely to face any lesser a challenge in this respect. Despite the infancy of nanotechnology applications for food, there are already demands for demonstrations that the new technological developments will have some real benefits for the consumer and not for the industry alone, and that the promised benefits will outweigh any risks to the consumer and/or the environment.

Like any new technology, public confidence, trust, and ultimately acceptance will be the key determinants for the success or failure of nanotechnology applications for food. Nanotechnology-derived food products will also be new to consumers, and it remains to be seen how they will be viewed by the general public. It is, nevertheless, obvious that uncertainties and lack of knowledge in regard to any new technology, or a lack of clear communication of the risks and benefits, can raise concerns amongst the public. In the present era of heightened consumer awareness, nanotechnology applications in the food sector seem to have already opened up a new debate amongst the stakeholders. There are, variously, calls ranging from a moratorium to an outright ban on the use of nanotechnologies for food. A recent report on the survey by the German Federal Institute of Risk Assessment[20] has shown that the current consumer opinion in the EU, whilst conducive to many nanotechnology applications, is not entirely favourable in regard to its use in food. This bears some resonance with similar issues of food irradiation and of genetically modified (GM) crops in the past, where a lack of clear demonstration of consumer safety and benefits resulted in a negative public response in many countries.

Public perception of a new technology is, however, influenced by an array of complex factors. In developed countries, where food is currently plentiful and affordable, there is a degree of public scepticism towards the food products that are (or perceived to be) unduly over-processed, or that lack wholesomeness, freshness or 'naturalness'. It also appears that even though food production is becoming increasingly globalised, public perceptions and priorities on food quality and safety do have more of a national characteristic, based partly on economic and cultural reasons. Thus, even within a single trading block, such as Europe, consumer priorities differ from country to country, some placing pesticides, for example, at the top of the agenda, some animal welfare, whilst others consider genetically modified organisms most worrying, etc. A similar heterogeneity in the perception and acceptance of nanotechnology is likely. Indeed, the public opinion in Europe seems to contrast with that in the USA. A survey carried out in 2008 for the Woodrow Wilson Institute for Scholars[21] has shown that, whilst a large majority of Americans has little or no knowledge of nanotechnology, the respondents expressed positive expectations when told about the potential benefits and risks of the technology. The consumer

perception of nanofood in less well-off parts of the world may also be different from that in the developed world. (The recently coined term 'nanofood' refers to the use of nanotechnology techniques, materials or tools for production, processing or packaging of food.)

In this regard, it is logical to think that some applications will be seen *per se* as less acceptable than others. These aspects have been discussed in detail in Chapters 2 and 3, and analogies have been drawn from experiences with other technologies introduced into the food sector in the past.

1.4 Natural Nanostructures in Food

Whilst nanotechnologies offer exciting opportunities for the development of new tastes and textures through the development of nanostructures, emulsions and micelles in foodstuffs, it is known that our food already contains certain natural nanostructures. The three basic food constituents are proteins, carbohydrates and fats. Many food proteins and carbohydrate starches exist naturally in the nanoscale and simple triglyceride lipids are about 2 nm long. Food substances are also metabolised in the body at a nanoscale. Although proteins, carbohydrates and lipids are each digested in the gastrointestinal tract (GIT) in a different way, a common factor is that they are all broken down to nanostructures before assimilation. It has, therefore, been argued that our body is already used to dealing with nanostructures in the GIT, and that foods processed at the nanoscale would simply be more readily digestible, absorbed and bioavailable in the body. However, it remains to be seen whether nanoscale processing of food materials might produce structures that are different from those that occur naturally. These aspects are discussed in more detail in Chapter 4.

1.5 Potential Benefits and Market Drivers

Like any other sector, the food industry is also driven by innovations, competitiveness and profitability. The industry is, therefore, always seeking new technologies to offer products with improved tastes, flavours, textures, longer shelf-life, better safety and traceability. Other pressures, such as increased health consciousness amongst consumers and tighter regulatory controls, have also driven the industry to look for new ways to reduce the amount of salt, sugar, fat, artificial colours and preservatives in their products, and to address certain food-related ailments, such as obesity, diabetes, cardiovascular diseases, digestive disorders, certain types of cancer (e.g. bowel cancer) and food allergies. The needs for food packaging have also changed with time, to stronger but lightweight, recyclable and functional packaging materials. Food labels are now expected to provide much more than a mere list of ingredients and cooking instructions, and 'Smart' labels are finding an increasing use in monitoring food quality, safety and security during transportation and storage. Other 'newer' societal and technological pressures are affecting the food industry, such as the

need to control pathogens and certain toxins in food, to reduce the amount of packaging, food waste and carbon footprint in the life cycle of food products. In this context, the advent of nanotechnology has raised new hopes that it can address many of the industry's needs (Figure 1.3). These aspects are discussed in more detail in Chapters 5, 6 and 7.

A number of recent reports and reviews have identified the current and short-term projected applications of nanotechnology for the food sector.[4 7] Although such applications are relatively new and emergent, they appear to have started to make a global impact. A current niche for such applications is in the areas where there is an overlap between the food, medicines and cosmetics sectors. Many food products are marketed as a means to enhance nutrition for different lifestyles and age groups, and as an aid to health, beauty and wellbeing. This has resulted in certain hybrid sub-sectors that include nutritional supplements, health foods, nutraceuticals, cosmeceuticals and nutricosmetics. These hybrid sectors have so far been the first focus of nanotechnology applications, which have only recently started to appear in the mainstream food sector. Thus a large majority of the currently available nanotechnology products falls in the areas of supplements, health foods and nutraceuticals, with only a few products in the food and beverage areas. The main tenet behind the development of nano-sized ingredients and additives appears to be the enhanced uptake and bioavailability

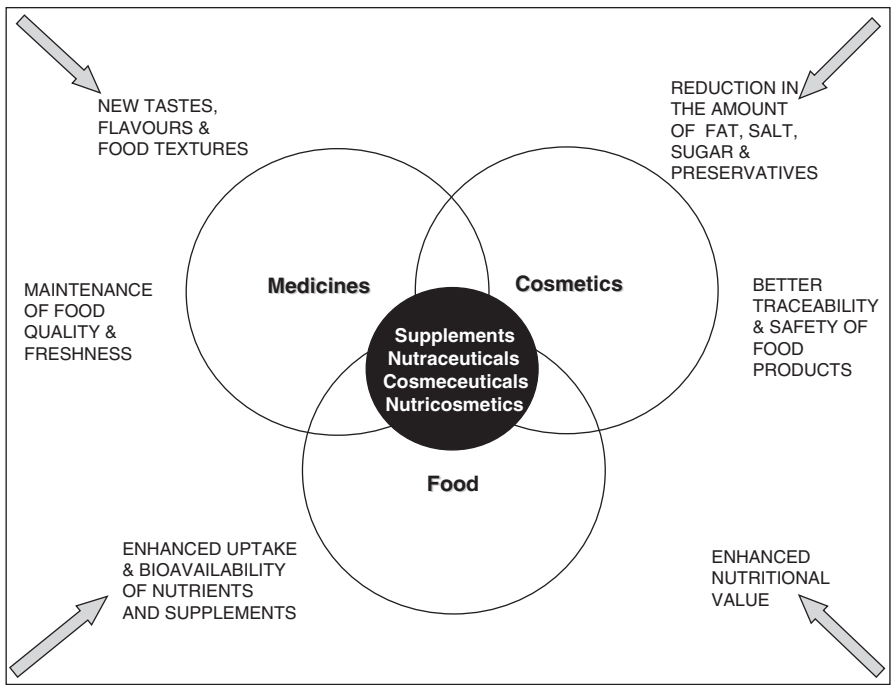

Figure 1.3 The main projected benefits of nanotechnology applications for food and related sectors.

of nano-sized substances in the body, although other benefits such as improvement in taste, consistency, stability and texture, etc. have also been claimed. A major current area of application for ENPs is in food packaging, in the form of innovative nanoparticle/polymer composites that offer improved mechanical or antimicrobial properties.

The number of companies undertaking research and/or using nanotechnology for food applications has been estimated to be between 200 (ref. 8) and 400 (ref. 9). These almost certainly include some of the major international food and beverage firms. However, accurate information on the true scale of industrial activity in this area is difficult to obtain because of commercial and other sensitivities. A number of major food corporations, who had been at the forefront of food nanotechnology R&D until a few years ago, now disown any involvement in this area. This has made it difficult to gauge the accurate level of commercial activity in this area. The absence of any quality scheme for nanofood products makes it even more difficult to segregate 'real' nano products from those that are based on unsubstantiated claims to project the 'magic' of nanotechnologies for short-term commercial gains. This has also raised concerns that at least some, if not many, of the products claimed to have derived from nanotechnology may in fact not be so. Conversely, some products may contain a nano component, but may not be claimed for its presence. In this context, some market forecasts for a dramatic future growth in the nanofood sector need to viewed with caution. It is, nevertheless, noteworthy that the number of nano (health)food products has been on a steady increase over the past few years. It is also likely that many more products and applications are currently in the R&D pipeline, and will appear on the market in coming years.

It is evident from available reports that the current nanofood sector is led by the USA, followed by Japan and China.[10] Despite the infancy of the nanofood sector, the overall size of the global market in 2006 has been estimated at between US$410 million (ref. 9) to US$7 billion (ref. 10). Future estimates vary between US$5.8 billion in 2012 (ref. 9) to US$20.4 billion by 2010 (ref. 10). Thus despite the current uncertainties, it appears that the upward trend in the nanofood sector will continue and may gather pace in the coming years.[5,9] The commercial exploitation of nanotechnology is also almost concurrent with that of the start of online marketing of consumer products through the internet. Thus virtually all of the currently available nanotechnology-derived consumer products can be bought by the consumer via the internet anywhere in the world.

1.6 Current and Projected Applications of Nanotechnology for the Food Sector

The applications of nanotechnology for the (health)food sector are potentially numerous, and are discussed in detail in Chapters 5 and 6. The main focus of developments has so far been on innovative food packaging, smart labels, nano-sized or nano-encapsulated ingredients and additives, and nanocarriers for delivery of nutrients and supplements.[5]

1.6.1 Innovative Food Packaging Materials

Whilst most nanotechnology applications for food and beverages are currently at R&D or near-market stages, the applications for food packaging are rapidly becoming a commercial reality.[5,9] A contributing factor to the rapid commercial developments in this area appears to be the expectation that, due to the fixed or embedded nature of ENPs in plastic polymers, they are not likely to pose any significant risk to the consumer. Nanotechnology applications for food contact materials (FCMs) already make up the largest share of the current and short-term predicted nanofood market.[9] It has been estimated that nanotechnology-derived packaging (including food packaging) will make up to 19% of the share of nanotechnology products and applications in the global consumer goods industry by 2015.[11] The main developments in the area of nanotechnology-derived FCMs include the following.

- 'Improved' FCMs in terms of flexibility, gas barrier properties and temperature/moisture stability. Typical examples include polymer composites with nanoclay (gas barrier), silicon dioxide (abrasion resistance), titanium dioxide (UV absorption) and titanium nitride (processing aid, mechanical strength). Also under research are nanocomposites of biodegradable polymers, such as nanoclay composites with polymers of starch and polylactic acid, for improved mechanical and moisture barrier properties.
- 'Active' FCMs incorporating metal or metal oxide nanoparticles (e.g. silver, zinc oxide, magnesium oxide) for antimicrobial properties. They are claimed to prevent microbial growth on the surface of plastics and hence keep the food within fresher for relatively longer periods.
- 'Intelligent' and 'Smart' packaging incorporating nano-sized sensors that can monitor the condition of the food during transportation and storage. Of particular interest in this regard are the safety and quality indicators that can be applied as labels or coatings to add an intelligent function to food packaging. These could, for example, monitor the integrity of the packages sealed under vacuum or inert atmosphere by detecting leaks, freeze-thaw-refreeze scenarios by detecting variations in temperature with time, or microbial safety by detecting the deterioration of foodstuffs.
- Nanocoatings for FCMs with barrier or antimicrobial properties, and for 'active' or self-cleaning surfaces in food processing facilities such as abattoirs.

The currently available FCMs include multi-layered PET bottles with nanoclay composite for gas barrier. The technology is understood to be already used by some large breweries. Other examples include food containers made of plastic/nano-silver composite and wrapping film containing nano-zinc oxide for antimicrobial protection of food. As mentioned before, market estimates for the current and short-term predicted applications suggest that nanotechnology-derived food packaging materials already make up the largest share of the overall nanofood market.[9] Chapter 6 covers the nanotechnology processes, products and applications for food packaging materials in detail.

1.6.2 Nano Ingredients and Additives

A key application area of nanotechnology for food processing is the development of certain nano-structured (also termed as nano-textured) foodstuffs, such as spreads, mayonnaises, creams, yoghurts and ice creams. The nano-structuring of food materials has been claimed for new tastes, improved textures, consistency and stability of emulsions, compared to equivalent conventionally processed products. A typical product of this technology could be in the form of a low-fat nano-textured product that is as 'creamy' as the full-fat alternative, and hence would offer a 'healthy' option to the consumer. Currently, there is no clear example of a proclaimed nano-structured food product that is commercially available, although some products are known to be at the R&D stage.[5] One such example under R&D is that of a mayonnaise which is composed of nanomicelles that contain nanodroplets of water inside. The mayonnaise would offer taste and texture attributes similar to the full-fat equivalent, but with a substantial reduction in the amount of fat intake by the consumer.

Another area of application involves the use of nano-sized or nano-encapsulated food additives. This type of application is expected to exploit a much larger segment of the (health)food sector, encompassing colours, preservatives, flavourings and supplements. The main advantage is said to be a better dispersability of water-insoluble additives in foodstuffs without the use of additional fat or surfactants, and enhanced tastes and flavours due to enlarged surface area of nano-sized additives over conventional forms. A range of consumer products containing nano-sized additives is already available in the supplements, nutraceuticals and (health)food sectors. These include minerals, antimicrobials, vitamins, antioxidants, etc. Virtually all of these products also claim enhanced absorption and bioavailability in the body compared to their conventional equivalents.

Nano-encapsulation is the technological extension of micro-encapsulation that has been used by the industry for (health)food ingredients and additives for many years. Nano-encapsulation offers benefits that are similar to, but better than, micro-encapsulation, in terms of preserving the ingredients and additives during processing and storage, masking unpleasant tastes and flavours, controlling the release of additives, as well as enhanced uptake of the encapsulated nutrients and supplements.

Following food packaging, nano-encapsulation is currently the largest area of nanotechnology applications in the (heath)food sector. Nano-encapsulation in the form of nanomicelles, liposomes or protein-based carrier systems has been used to develop delivery systems for additives and supplements in food and beverage products. A growing number of (health)food and nutraceutical products based on nanocarrier technology are already available on the market. These include a number of food additives and supplements. Other products containing nano-antimicrobials and nano-antioxidants, etc., are also commercially available. The concept of nanodelivery systems seems to have originated from research on targeted delivery of drugs and therapeutics. However, the use of similar technology in foodstuffs is interesting in the sense that whilst

it can offer increased absorption, uptake and bioavailability, it also has the potential to alter tissue distribution of the substances in the body. For example, certain water-soluble compounds can be rendered fat dispersible through nanocarrier technology. *Vice versa*, fat-dispersible compounds can be rendered water dispersible. It is hoped that these nanocarriers are completely broken down and their contents are released in the GI tract. As such, the encapsulated compounds will not be any different from their conventional equivalents. However, if a nanocarrier system is capable of delivering the encapsulated substance to the bloodstream, its absorption, tissue distribution and bioavailability may be drastically different from the conventional forms. This raises the concern that some nanocarriers may act as a 'Trojan Horse' and facilitate translocation of the encapsulated substances or other foreign materials to unintended parts of the body. The currently known and anticipated nanotechnology applications for food ingredients, additives and supplements are described and discussed in more detail in Chapter 5.

1.6.3 Other Applications

The apparent benefits of substituting active ingredients or carriers with nano-sized equivalents has also opened up the doors for research into potential applications of nanotechnology to pesticides, veterinary medicines, and other agrochemicals such as fertilisers and plant growth regulators. The anticipated benefits, driving R&D in these areas, include a potential reduction in the use of certain agrochemicals, and a better ability to control application and dosage of active ingredients in the field. Nano-encapsulated materials and solid lipid nanoparticles have also been explored for the delivery of certain agrochemicals, including the slow- or controlled-release fertilisers and pesticides. However, despite a growing industrial interest in this area, examples of any available products are virtually non-existent. Some R&D has been reported into the potential use of nano-emulsions, micronised (volcanic) rock dust, and nano-silica as a delivery system for pesticides, fertilisers and growth regulators.

In theory, the nano-sized supplements, e.g. vitamins and minerals, developed for human (health)food applications can equally be used for animal feed. Some feed-grade nano-vitamin mixes are also available for use in poultry and livestock feed. Examples of the nano-sized additives specifically developed for animal feed include a natural biopolymer from yeast cell wall that is intended to bind mycotoxins to protect animals against mycotoxicosis,[12] and the possible use of an aflatoxin binding nano-additive for animal feed, which is derived from modified nanoclay.[13] Another interesting example of R&D in this area is 'intelligent chicken feed', which is reported to contain polystyrene nanoparticles coated with host proteins to mimic the host cell surface. When used in chicken feed, these nanoparticles are reported to offer large surface areas that can bind and purge the animal of targeted pathogens without the use of antibiotics.[14]

1.7 Potential Health Effects

The rapid proliferation of nanotechnologies into consumer products, especially food, has raised a number of concerns over their safety to the consumer. These concerns, however, seem to arise mainly from the current lack of knowledge in regard to the potential effects and impacts of ENPs on human health and the environment, and from the (perceived) lack of appropriate regulatory controls. These aspects are discussed in detail in Chapter 8.

It is known that the conventional physicochemical rules are not fully applicable at the nanometer scale, and that there can be some fundamental shifts in the physicochemical properties, behaviour and interactions of ENPs compared to their bulk equivalents. For example, quantum effects may have a much greater influence on the properties of ENPs in the lower nanometer size range. In some cases, such changes in physicochemical properties could lead to a change in the effects and impacts on biological systems. Some studies have already suggested a deviation in the toxicity profile for some ENPs compared to conventional equivalents. The use of insoluble (or indigestible) ENPs in food applications, such as certain metal(oxide) ENPs, that are unlikely to be assimilated inside or outside the GI tract, raises a number of consumer safety concerns. The likelihood of translocation of such ENPs with large, and potentially reactive, surface areas to various cells and tissues in the body may pose certain risks to consumer health. Thus whilst a relatively small quantity of an ENP can provide a similar level of functionality that would require a much greater quantity of the bulk equivalent, by the same token it may have a proportionately greater impact on human health and/ or the environment. ENPs are also known to adsorb or bind various compounds and moieties on their surfaces and, as discussed before, may act as a carrier of potentially harmful contaminants and foreign substances into the blood and facilitate their distribution to different organs and tissues in the body.

Another important aspect to consider in relation to potential harmful effects of ENP is their ability to penetrate cellular barriers. This adds a new dimension to particulate toxicology, as ENPs can potentially reach new targets in the body where the entry of larger particulates would be restricted.

Depending on the surface chemistry, ENPs can interact with various chemical and biological entities and such interactions may have a substantial effect on the distribution and excretion of an ENP. In this regard, there is emerging evidence to suggest that ENPs may become surface-coated with certain biomolecules, especially proteins, and such coatings can direct them to specific locations in the body.[15] This suggests that ENPs can undergo complex interactions in biological environments. It is also likely that the ENPs added to foodstuffs will undergo certain transformations that may affect their translocation, bioavailability and toxicity in a biological system. Whilst nothing can be generalised at this stage due to the limited nature of the available knowledge, a predominant manifestation of ENP exposure in biological systems has been shown to be an increase in the generation of reactive oxyradicals. Dependent on the level and duration of the exposure, an increased oxyradical generation may

lead to oxidative stress and inflammatory reactions. A greater uptake, absorption and bioavailability of certain nano-sized food additives (such as preservatives) may also lead to certain health consequences. Certain metal (oxide) ENPs are known to have strong antimicrobial activity. However, it is not known how their intake via food and drink might affect the gut natural microflora.

It is worth stressing that any risk to a consumer of nanofood will depend on a number of factors, such as the concentration of an ENP in a given food product, the amount and frequency of consumption of the product and, more importantly, the physicochemical nature, uptake, translocation and bioavailability of the ingested ENPs. Currently, there are major knowledge gaps in regard to the behaviour, interactions, fate and toxicological effects of ENPs inside and outside the GI tract. It is likely that most ENPs added to food will not remain in a free form (and hence not be available for translocation) because of agglomeration, binding with other food components or reaction with stomach acid or digestive enzymes. It is also important to note that much of the current evidence indicating harmful effects of some ENPs either relates to inhalation exposure, or is based on *in vitro* assessments. Thus the full extent of hazard, exposure and risk from the ingestion of ENPs via food and drink are largely unknown. In anticipation of the likely developments in the nanofood sector, however, it is imperative that the safety of nanotechnology-derived products is addressed adequately, so that whilst the new developments bring benefits to the consumer, they are also safe to human health and the environment.

1.8 Potential Health Risks and Governance of Risks

The main likely route of entry of micro- or nano-sized particles to the gut is through consumption of food and drink, although some entry through clearance of the lungs is also possible. A healthy digestive system allows absorption of substances (such as nutrients) from the gut only after digestion of foodstuffs. The gut wall is thus designed to ensure the passage of dietary nutrients, but prevent larger or foreign materials. The very small size of nanofood ingredients and additives may give them a greater ability to cross the gut wall. The resulting increase in absorption and bioavailability could give rise to higher internal exposure, with higher plasma concentrations (from a higher absorption rate), or higher area-under-the-curve exposure (from higher uptake efficiency). From these, a number of possible consumer health implications from the consumption of nanofoods may be envisaged. For example, this may lead to an altered nutrient profile in the body due to greater absorption of certain nano-ingredients, or increased health consequences due to a greater absorption of some nano-additives (such as preservatives).

It is well documented that the GI uptake of exogenous nanoparticles is greater than microparticles.[16] Translocation through the gut epithelium would also be dependent on the physiochemical properties of an ENP, e.g. size, surface charge,

hydrophobicity, surface chemistry, *etc*. It will also be affected by physiology of the GI tract. For example, translocation may be different in a diseased rather than a normal healthy gut.[16] It has also been speculated that the presence of particulate materials in the diet can exacerbate certain aliments, such as Crohn's disease and irritable bowel syndrome (IBS). Trials carried out so far to test whether a reduction of microparticles in the diet can reduce the symptoms of Crohn's and IBD have, however, produced contradicting results and it is uncertain whether the presence of micro- or nano-particles is unequivocally linked to initiation of the diseases. These aspects are discussed in detail in Chapters 8, 9 and 11.

The consumer risk from the use of food or drinks packaged in nano-technology-derived FCMs would be dependent on the migration of ENPs into foodstuffs. Such migration data have so far been very limited, but recent studies have shown insignificantly low levels of migration of ENPs in food contact materials,[22] (also Bradley *et al*., unpublished). The presence of ENPs also did not seem to affect the migration of other non-nano components. Thus, whilst more testing is needed to ascertain migration patterns in other ENP/polymer composites, it seems that this application area of nanotechnology may not carry a significant risk of ENP exposure for consumers.

1.9 Adequacy of Regulatory Frameworks

The rapidly expanding nanotechnological developments in a wide range of industrial sectors are also posing a challenge to the current regulatory frameworks. In this regard, questions have already been raised whether the current risk assessment (RA) paradigm, designed for conventional substances, is applicable and adequate for nanomaterials. It is evident from recent regulatory reviews that, at present, there is no nano-specific regulation anywhere in the world. Furthermore, there is a lack of specific guidelines, guidance documents for testing, or testing requirements under any of the existing regulations that relate specifically to ENPs in terms of size or other distinct physicochemical properties. Studies have, however, regarded the existing models for risk assessment applicable to materials and products of nanotechnology, highlighting the need for certain modifications in testing methodologies. There are also major knowledge gaps in relation to the effects of most ENPs on human health, agreed dose units for hazard and exposure assessments, and reliable and validated methods for measurement and characterisation of ENPs in complex food matrices. Despite such uncertainties, the new nanotechnological developments are not taking place in a regulatory void, as the potential risks will be controlled under the existing frameworks. In this regard, a number of vertical regulations that relate to specific processes, materials, products or applications and horizontal regulations are relevant and applicable.

Studies carried out to identify potential regulatory gaps[5,17,18] have highlighted certain uncertainties and inadequacies in the existing regulatory frameworks in relation to the use of nanotechnologies in food. For example,

although ENPs are likely to be covered under the new chemicals regulation (REACH) in the EU, there is currently no special provision to consider them differently from the conventional equivalents, and the threshold of 1 tonne/year set out under REACH may be too high for some ENPs.[18]

Furthermore, although most nanotechnology applications would come under some form of approval process, the existing food legislation in the EU does not differentiate between 'conventional' and 'nano' forms of already approved additives. There are, nevertheless, recent regulatory proposals to address these uncertainties. These aspects are discussed in detail in Chapter 10.

1.10 Conclusions

A cursory look at the new technological developments shows that nanotechnologies offer some real and wide-ranging benefits to the whole of the food chain. Examples of these include new tastes, textures, sensations and consistencies of food products; potential reduction in the amount of fat, salt and other additives; enhancements in the absorption and bioavailability of nutrients and supplements; preservation of food quality and freshness, and better traceability and security of food products through innovative packaging applications. It is also clear from the current and projected applications in the (health)food sectors that they have been on a steady increase worldwide. Currently, food packaging applications make up the largest share of nanofood market, followed by nano-sized and/or nano-encapsulated ingredients and additives for (health)food applications. A number of nanotechnology-derived ingredients, additives, and FCMs is already available worldwide. However, such technological developments are still new or emergent in the EU and many other countries, where there is only a marginal level of current applications. Considering the global nature of food business, and that several companies and research institutions are currently exploring new possible applications in the food and related sectors, it is not unreasonable to expect that nanofood products will be available to the consumer in an increasing number and variety in the coming years. The market penetration of such products in different countries and regions will depend on, amongst other factors, the price and quality of the products. This also means that there will be a growing need for strategies to regulate the risks, and establishment of liabilities, at the global level. This will inevitably pose a bigger challenge for the regulatory authorities because food laws in different countries may not conform to each other. In due course, such issues are likely to be resolved through the development of global frameworks that relate to key international trade agreements, such as those administered by the World Trade Organization.[19]

Nanotechnology applications for food and health food sectors have undoubtedly opened up enormous opportunities for innovation and new developments, but at the same time have also raised new challenges in regard to ensuring the consumer safety and in communicating the risks and benefits without jeopardising the pace of the new technological developments. In this

regard, the industry is likely to face certain immediate challenges. These relate to demonstrating the clear benefits of nanofood, ensuring a stringent quality control of the products, regulatory compliance and providing health and safety reassurance to the consumer. This book is aimed at providing the much needed insight to the various aspects and issues in relation to the new and exciting technological developments that nanotechnologies are offering to the food and related sectors.

References

1. R. Feynman, *There's Plenty of Room at the Bottom*, 1959, available at www.its.caltech.edu/~feynman/plenty.html.
2. *British Standard Institute's Publicly Available Specification PAS 136: 2007 'Terminology for Nanomaterials'*, 2007, available at: www.bsi-global.com/en/Standards-and-Publications/Industry-Sectors/Nanotechnologies/PAS-136/Download-PAS-136/.
3. IGD, *Global Retailing Report*, 2005, available at www.igd.com/CIR.asp?menuid=50&cirid=1505.
4. H. Bouwmeester, S. Dekkers, M. Noordam, W. Hagens, A. Bulder, C. de Heer, S. ten Voorde, S. Wijnhoven and A. Sips, *Health Impact of Nanotechnologies in Food Production. Report 2007.014*, published by RIKILT – Institute of Food Safety, Wageningen UR and National Institute of Public Health & the Environment – Center for Substances and Integrated Risk Assessment, 2007.
5. Q. Chaudhry, M. Scotter, J. Blackburn, B. Ross, A. Boxall, L. Castle, R. Aitken and R. Watkins, *Food Addit. Contam.*, 2008, **25**, 241.
6. EFSA (European Food Safety Authority), *Draft Opinion of the Scientific Committee on the Risks Arising from Nanoscience and Nanotechnologies on Food and Feed Safety, Endorsed for public consultation*, 2008.
7. FSAI (Food Safety Authority of Ireland), *The Relevance for Food Safety of Applications of Nanotechnology in the Food and Feed Industries*, Food Safety Authority of Ireland, Abbey Court, Lower Abbey Street, Dublin 1, 2008.
8. IFST (Institute of Food Science and Technology), *Nanotechnology Information Statement*, 2006, available at www.ifst.org/uploadedfiles/cms/store/attachments/nanotechnology.pdf.
9. Cientifica Report, *Nanotechnologies in the Food Industry*, 2006.
10. Helmut Kaiser Consultancy, *Study: Nanotechnology in Food and Food Processing Industry Worldwide 2003–2006–2010–2015*, 2004, available at www.hkc22.com/Nanofood.html.
11. Nanoposts report, *Nanotechnology and Consumer Goods – Market and Applications to 2015*, published by Nanoposts.com, 2008.
12. Specialty Biotech Thailand: www.sbtthai.com/profile.html.
13. S. YingHua, X. ZiRong, F. JianLei, H. CaiHong and X. MeiSheng, *Sci. Agric. Sinica*, 2005, **38**(5), 1069–1072.

14. *Nanotechnology for Intelligent Bird Feed*, available at www.allaboutfeed.net/news/id102-40104/nanotechnology_for_intelligent_bird_feed.html.
15. I. Lynch and K. A. Dawson, *Nano Today*, 2008, **3**, 40.
16. A. des Rieux, V. Fievez, M. Garinot, Y. J. Schneider and V. Preat, *J. Controlled Release*, 2006, **116**, 1.
17. Q. Chaudhry, J. Blackburn, P. Floyd, C. George, T. Nwaogu, A. Boxall and R. Aitken, *A Scoping Study to Identify Regulatory Gaps For The Products and Applications of Nanotechnologies, Central Science Laboratory, York*, 2006, available at: www.defra.gov.uk/science/Project_Data/DocumentLibrary/CB01075/CB01075_3373_FRP.doc.
18. Q. Chaudhry, C. George and R. Watkins, in *New Global Frontiers in Regulation: The Age of Nanotechnology*, ed. G. Hodge, D. Bowman and K. Ludlow, Edward Elgar, Cheltenham, 2007, ch. 11.
19. G. Hodge, D. Bowman and K. Ludlow, (Eds.), *New Global Frontiers in Regulation, In The Age of Nanotechnology,* Edward Elgar, Cheltenham, 2007.
20. R. Zimmer, R. Hertel and G.-F. Böl, (eds.), *Public Perceptions about Nanotechnology – Representative Survey and Basic Morphological-Psychological Study*, Federal Institute for Risk Assessment, Berlin, 2008, ISBN 3-938163-43-7.
21. Peter D. Hart Research Associates, Inc (2008), *Awareness of and Attitudes Towards Nanotechnology and Synthetic Biology*, Washington, DC, www.nanotechproject.org/process/assets/files/7040/final-synbioreport.pdf.
22. P. Šimon, Q. Chaudhry and D. Bakoš, *J. Food and Nutrition Research*, 2008, **47**(3), 105.

CHAPTER 2

The Evolution of Food Technology, Novel Foods, and the Psychology of Novel Food 'Acceptance'

LYNN FREWER AND ARNOUT FISCHER

Wageningen University, Marketing and Consumer Behaviour Group, P.O. Box 8130, 6700 EW, Wageningen, The Netherlands

2.1 Introduction

Food availability across the globe is the result of a complex interaction between traditional and evolving agricultural practices (for example, the development of new crops and agricultural practices and the introduction of novel technologies to agri-food production), market demand and economic drivers, environmental factors (such as the impact of climate change on food productivity or emerging diseases within the food production chain) as well as the preferences of consumers within different cultural contexts and with different degrees of income potentially available to spend on food. Despite the 'green revolution' in agricultural production practices, which occurred in the middle of the 20th century, food availability still presents a problem in some parts of the world. In other regions, over-consumption has a bigger impact on public health. The issue of food security is also unpredictable. For example, at the time of writing, the 'biofuels crisis'[1,2] has led to an increase in food prices internationally, as well as protests in some regions of the globe associated with both unaffordable foods

RSC Nanoscience & Nanotechnology No. 14
Nanotechnologies in Food
Edited by Qasim Chaudhry, Laurence Castle and Richard Watkins

Published by the Royal Society of Chemistry, www.rsc.org

and reduced food availability.[3] Specifically, the increase in agricultural land use for biofuels has reduced the existing land available for food production. At the same time, the increase in demand for animal proteins, in particular in emerging economies such as China and India, has increased demand for commodity products such as grains.[4,5] The biofuels issue provides an example of where the need for agri-technology innovation has provided unpredicted secondary effects in the agri-food sectors, which has subsequently interacted with consumer demands and concerns to create further problems associated with the food supply (in this case, food security and affordability).

Technological innovation in the area of food production has a long and complex history. Technological advances in the agri-food sector have occurred since prehistoric times. For example, domestication of plants is thought to have occurred at the end of the Pleistocene era as a consequence of human responses to climatic instability and the colonisation of suitable habitats by humans. Wild plants were selected, gathered and interbred by human 'hunter-gatherers' in order to improve food availability and to meet changes in demand from more static and permanent communities that were then being established .[6] In order to maintain a more secure supply of food, humans expanded their diets to include more complex preparation of otherwise inedible or indigestible foods and food ingredients, and the intentional cultivation of cereal products for human and animal consumption.[7] It is argued that climate change reduced the availability of foods, which in turn triggered the introduction of crop cultivation technologies to meet this shortfall.[6]

The introduction of agricultural implements and tools also increased food availability through the implementation of more efficient production processes. These reduced the intensity of labour required to produce a given amount of food, freeing up human resources for other activity. For example, the introduction of the mouldboard plough in medieval Europe[8] allowed the European population to recover from the period of scarcity and population reductions after the collapse of the Roman empire from the 10th century and led to population growth during the Middle Ages. Other technological innovations have continued to influence agricultural practices, improving food availability, as well as safety and quality and, increasingly, the nutritional content of foods and ingredients. The introduction of mechanised farming in the 19th century, which increased the rate and amount of food production, also drove a population shift from rural to urban environments. This innovation was accompanied by farm worker protests in the form of the Luddites, who were concerned about changes in traditional employment and ways of living.[9] However, the term 'Luddite' is now commonly used to refer to any individual or group who opposes technological innovation, independent of whether the introduction of the new technology or technologically driven has an impact which extends beyond that immediately associated with the technology itself.

The early 20th century was associated with the development and increased use of chemical fertilisers and pesticides. The international application of such intensive farming methods was termed 'the green revolution' and occurred in the middle and latter half of the 20th century.[10] At the same time, societal

concerns about the negative effects of such agrochemicals on human health and the environment also grew, signalled by the publication in 1962 of *The Silent Spring*[11] by Rachel Carson, a seminal book which contributed to the development of the environmentalist movement in the latter half of the 20th century. More recently, concerns have been expressed about the replacement of natural ecosystems with novel agricultural practices driven by technological innovation (for example, the destruction of natural rainforest and replacement by monocultures). Other societally controversial applications of technology in the agri-food sector have included the introduction of irradiated foods[12,13] and the development and introduction of genetically modified foods and crops.[14]

Of course, societal negativity to technological innovation is not unique to the agri-food sector. For example, citizen protest and mobilisation against nuclear energy has been observed since the 1950s.[15] Nevertheless, there does seem to be considerable and domain-specific societal sensitivity towards the use of technology in food production, as compared to medical or pharmaceutical developments, for example. These sensitivities must be addressed if technological innovation in this sector is to be successful.

From a psychological perspective, food is of particular interest as it combines the most basic human need for survival with other factors including hedonistic response to particular tastes and textures, cultural determinants of specific food preferences and regional and temporal variation in what is considered to be a 'healthy' or 'risky' food.[16] Food considered appropriate in one culture or society might be inappropriate, a subject of cultural taboo, or even illegal, in others. There is extensive variation regarding culture-specific rules and practices about how food is produced, prepared, presented and eaten.[17] These , in turn, influence peoples perception of risk and benefit associated with different kinds of foods, as well as the food production technologies used to produce them. In this context, consumer food choices related to emerging agri-food technologies serve to illustrate many of the relevant issues pertinent to the introduction and application of emerging technologies into society more generally.

Although specific and localised consumer concerns about food technologies have been observed historically, consumer preferences regarding agri-food production processes are generally regarded as most forcefully originating in the 1960s, and becoming more intense from the 1990s onwards.[18] Consumer choice is an important concept in food selection by consumers, and may militate against the application of technologies to food production. For example, many food products are still produced using long established methods, illustrating consumer preferences for the use of traditional production methods as applied to food production.[19] The societal demand for such traditional approaches to food production is illustrated by the introduction of authenticity labelling by institutions such as the European Commission. Similarly, perceived 'naturalness' is a factor which appears to be valued by consumers in the context of food choice.[20] As a consequence, novel foods, or the new technologies used to produce them, may be rejected by consumers as being 'unnatural' or 'untraditional'. Differences in consumer variation in the

acceptance of technology in the agri-food sector may be prone to cross-cultural variation. Nonetheless, stable differences between societies have been observed which potentially determine the roles played by companies, governments and consumers in different cultures and economic systems that shape the international pattern of the food economy, and have specific implications for the introduction of novel food technologies.[21]

It has been established that, in general, people tend to be more accepting of emerging technologies that are applied in the pharmacology and medical sectors than in the food sector. Functional foods (foods containing compounds with beneficial human health effects over and above those provided by the basic nutrients, minerals and vitamins) provide additional benefits to those provided by a 'traditional' food product, and are available to consumers as a consequence of recent advances in food technology such as genetic modification or, potentially in the near future, nano(bio)technology. The question arises as to whether consumers will accept products with additional health advantages if they are produced through application of a controversial new food technology.[22] The empirical information needed to resolve this issue is equivocal. For example, individuals allergic to birch pollen appear willing to accept the planting of genetically modified hypo-allergenic birch trees. In contrast, food allergic consumers are less willing to accept hypoallergenic foods produced using the same process.[23] To some extent, consumer belief in the health benefits of functional foods appears to be an important determinant of acceptance.[24] For example, Bech-Larsen and Grunert[25] report that consumer acceptance of functional foods is only moderately predicted by consumer concerns about the 'manipulation of nature'. However, it seems most likely that consumer acceptance of specific functional foods will be predicted by an interaction between consumer evaluation of the benefits associated with specific products and their concerns about the technology used to produce the food. From this, one might predict that the novel applications of nanotechnology in the agri-food sector will be dependent on a complex interaction between consumer evaluations of the perceived benefits and risks associated with novel products, as well as the production process used to produce them.[14]

2.2 A History of Consumer Risk Perception

Seminal research by Paul Slovic and colleagues identified the relevant psychological factors that determine responses to a particular hazard, as well as demonstrating that these also differ from expert assessments of the same risks.[26–28] Consumer risk perceptions in general, and those related to food risk in particular, appear to differ from those provided by individuals with 'technical' skills and knowledge about a specific hazard domain. In particular, early studies focused on understanding the risk perception of lay people resulted in different psychological dimensions of perceived risk being identified.[26,27,29,30] Specifically, it was found that factors that are not explicitly addressed as part of technical risk estimates may influence the perception of a given risk. This includes the extent to

which a risk is perceived to be unnatural, potentially catastrophic, or to which an individual perceives their own exposure to be involuntary. These psychological dimensions have proven to be reliable predictors of people's responses to potential risks associated with hazards across different hazard domains. It should also be noted that 'expert' responses to consumer concerns have frequently been contextualised by the observation that consumers accept exposure to potentially 'technical' risks whilst expressing concern about those that are technically 'small'. For example, the continued consumer consumption of unhealthy foods, such as those containing high levels of saturated fat, whilst simultaneously rejecting foods using highly technological production processes, has resulted in expert 'outrage' to the failure of consumers to respond to 'expert' advice. Much empirical research has focused on understanding why consumers reject technological production methods and novel foods, whilst at the same time attempting to understand the psychological basis of unhealthy food choices.

Following on from this, a body of scientific literature exists which describes empirical investigations into consumer perceptions of the risk associated with food, food-related hazards and food production technologies.[31 38] Various conclusions can be drawn from this research. For example, in addition to the more generic factors which determine risk perception, concerns may be very specific to particular hazard domains, including that of food and food production technologies.[22] In the area of food and technology acceptance, the perception that a particular technology may potentially have a negative impact on nature, or compromise important values such as environmental protection or animal welfare, may override the relevance of technical risk assessments in determining consumer responses.[33] This has been observed with genetically modified foods. Other psychological factors also influence consumer acceptance of emerging agri-food technologies. For example, some consumers appear to be especially neophobic in their response to novel foods, an effect that does not necessarily extend to all areas of technological innovation.[31,39,40] Food neophobia may have evolved in order to protect people from consuming potentially toxic new foods encountered during daily life,[41] but may also result in aversion to the novel foods of emerging technologies. There is evidence that neophobia is generally greater for older people (although this may be a cohort effect) and reduces as educational level increases.[40] Another issue that may contribute to the rejection of novel foods is emotional response.[41 43] The human emotional (and self-protective) response to contaminated food which may have evolved historically is that of disgust. This is a strong emotion that prevents consumption or acceptance of foods which are contaminated (for example through chemical contact or biological decay) or not familiar to the person consuming them.[43] Rozin *et al.*[42] argue that disgust is composed of three emotional dimensions:

1. the perception that a particular food will be incorporated in the body through oral contact
2. the food is perceived to be offensive and
3. the food is perceived to contaminate the body though the act of consumption.

The concept of disgust may be invoked by events, processes or associations that have little or no direct relation to actual food contamination[44] and is linked to avoidance behaviour or even physiological responses such as vomiting or an experience of nausea. If an agri-food technology is seen as unnatural or 'offensive' then negative consumer affective responses, such as neophobia or disgust, might be applied to associated products. In addition, coupling the production technology with images that evoke negative emotions may similarly invoke such affective responses.

Risk perceptions and other salient psychological factors have not always been taken into account in discussions associated with the development and commercialisation of new food technologies. These discussions have frequently been conducted in communities primarily consisting of experts drawn from the natural and technological sciences. As has been noted, expert groups have criticised negative consumer attitudes towards some food technologies, (for example, genetic engineering, food irradiation and pesticide use), while failing to consider the origins of these consumer attitudes. The behaviour of consumers in relation to food safety issues can, however, only be properly understood if there is a systematic understanding of the way in which consumers perceive risks, and indeed benefits, and how these relate to an effective food safety and technology development, risk management and commercialisation strategy.[45]

2.3 Consumer Acceptance of (Bio)nanotechnology in the Agri-food Sector

Following on from the example of the introduction of other emerging food technologies, the consumer and societal acceptance of agri-food nanotechnology will be very dependent on the delivery of tangible benefits to individual consumers and society more generally. Potential benefits may include improved nutrition (for example, more effective delivery of micronutrients), improved food quality (for example, extended shelf life or enhanced hedonic qualities), safer food (for example, prevention or detection of microbial or toxicological contamination) or more sustainable production (for example, reduced requirements for pesticides or irrigation, or improvements in animal health). For novel foods to be accepted, consumers must perceive that any potential benefits outweigh potential risks or negative effects (for example, potential for negative impact on the environment, human and animal health, or ethical concerns such as animal welfare or social equity). Although many improvements in food safety of traditional products have been achieved in recent decades, microbial, toxicological and carcinogenic substances are still sometimes found in products destined for human consumption.[46] Human health may be compromised by inappropriate nutrition linked to dietary choices or over-consumption of specific food components. In order to reduce the 'negative' effects of food production, novel foods have been developed in order to address health and environmental problems (for example, through production

of plants with advantageous traits, or animals which have a reduced impact on the environment as a result of intensive farming practices). Various applications have been, or are being, developed through application of genetic modification or, more recently, (bio)nanotechnology, which confer benefits in terms of human nutrition and micronutrient delivery, bio-security and development of plant or varieties that can grow in hostile environmental conditions, are resistant to pests or pesticides, or which have other desirable qualities such as improved aesthetic presentation. However, the introduction of new products and technologies may also introduce new hazards to the food chain, such as allergic reactions to novel proteins[47,48] or unexpected negative environmental impacts, such as the negative effects of chlorofluorocarbons (CFCs) on the ozone layer.[49] Even among experts, uncertainty associated with risk/benefit judgements may exist in the context of consumer protection and environmental impacts of new technologies.[50]

In summary, consumer perceptions associated with the introduction of novel food technologies are characterised by a range of specific perceptions, as well as those which can be generalised to technological innovation in general.[51] It is arguable that the agri-food sector may be potentially vulnerable to consumer concerns associated with the introduction of novel technologies, and that there is no reason to assume (bio)nanotechnology will not raise similar societal concerns unless lessons regarding effective development and commercialisation strategies are identified from historical precedents.

2.4 The Psychology of Food Choice: Implications for Emerging Food Technologies

Even within the agri-food sector, differences in risk perceptions have been identified that are associated with lifestyle hazards on one hand, and technological food-related hazards on the other.[52,53] For example, 'optimistic bias' is a psychological effect linked to risk perception,[54] whereby consumers perceive that they are at less risk than a member of society with whom they compare their own risks. Optimistic bias is more commonly observed for lifestyle food-related hazards, where people perceive they have higher levels of personal control over hazard exposure compared to more vulnerable, and less knowledgeable, individuals living in the same cultural context. Optimistic bias tends to disappear when the potential hazards are technological in origin, and hence are perceived to be less amenable to personal control.[55] However, repeated exposure of an individual to a potentially hazardous situation leads to a strong and stable risk attitude, which is not easily changed by risk communication.[56] It is possible that optimistic bias arises under circumstances when repeated exposure to the hazard under consideration does not immediately lead to negative consequences for the individual exposed, and people perceive they have a high level of personal control over their exposure. Once an optimistic bias has formed, it appears to be relatively stable. For example, there is evidence that consumers who have actually suffered from a food-induced illness

show only a temporal diminishing of their optimistic bias regarding their personal risks associated with microbial food safety.[57] However, in many 'lifestyle' situations, (for example, consumption of foods, over which an individual has a high perceived level of personal control but which may also be associated with a negative impact on health), optimistic biases result in consumers underestimating their personal risks from a particular hazard.

In contrast to their reactions to 'lifestyle' hazards, consumers may react negatively to the introduction of specific food technologies such as food irradiation, genetic modification of foods and nanotechnology applied to food production in such a way that consumers over-estimate the risks associated with hazards compared with the estimates provided by experts. Consumers have been observed to exhibit a much reduced, or indeed negative, optimistic bias associated with food technologies, possibly because they perceive a low level of personal control over exposure to potential risks. In general, consumers appear more concerned about risks which are related to the development and application of technology in comparison to naturally occurring risks, even when there is an equal probability of harm to human health.[58]

2.5 Persuasion and Attitude Change: Influencing Technology Acceptance?

Theories of persuasion have been developed in order to understand why, and under what circumstances, information may change people's attitudes regarding a particular issue, and to understand why differences in persuasion may occur between individuals and information domains. That is, theories of persuasion explain why not all participants in all situations react the same way to persuasive argumentation.

Dual process models of attitude change have attempted to explain the situational and contextual circumstances under which people change their attitudes following presentation of relevant information. It is now generally accepted that cognitive effort is required to process information in the in-depth and thoughtful way (systematic or elaborate processing) that represents the prerequirements of attitude change.[59] An individual needs to be motivated in order to expend this cognitive effort in scrutinising information relevant to attitude change. At the same time, an individual must also possess, and be willing to expend, the mental resources to actually process the relevant information. Some people are relatively highly motivated, compared to others, to engage in a challenging intellectual task, and tend to be intrinsically motivated to engage in *elaborate information processing* involving intense cognitive effort. Such individuals are regarded as possessing a personality trait that results in a high 'need for cognition'.[60]

As need for cognition tends to be higher among highly educated people,[61] one might predict that the developers of new and highly technological innovation, such as genetic modification or agri-food nanotechnology applications, may overestimate the *intrinsic motivation* of many members of the public to

process information provided about a technology and the associated risks through adoption of such an 'in-depth' information processing approach. This is partly because experts in a particular area are more personally motivated to process information relevant to their own personal interests, and partly because experts are more likely to be highly educated and themselves possess a 'high need for cognition'. However, under circumstances where individuals have only limited cognitive effort available to process the information, *heuristics* (or decision rules) and other mental 'shortcuts' in reasoning may be applied by an individual in order to reduce the effort which needs to be expended during the course of the information-processing task. This type of information processing is termed 'heuristic processing'. When heuristic processing is applied, attitude changes are less predictable and stable compared to situations where elaborate or systematic processing has been applied. Modern versions of the elaboration likelihood model[62] and the heuristic-systematic model[63] assume that elaborate and heuristic processing may occur simultaneously.

One of the reasons why consumer perceptions differ from those of experts may be the differential use of heuristics in processing information about a particular topic, especially those that require a great deal of cognitive resource to process. Attempts to 'educate' the public about the technical risks and benefits of a particular technology may therefore be unsuccessful, as lay people are unwilling and unmotivated to process complex information, rather resorting to 'heuristic' processing instead. Thus simply providing members of the public with technical information about the safety of a new technology and its products is unlikely, in itself, to be persuasive of the merits of the same technology.

If we assume that consumer acceptance or rejection of new food technologies should be based on the best and most balanced information available, elaborate or systematic processing on the part of consumers should to be the dominant path of information processing applied by individuals. If information can be made highly relevant to the person receiving the information, an individual's motivation to process it in an elaborate way will be increased.[64] A successful approach may be to design the information in such a way that a heuristic cue communicates the personal relevance to the recipient of the information, providing the motivation to engage in elaborative processing. However, if such approaches are to be adopted in risk/benefit communication about (for example) the use of nanotechnology in the agri-food sector, it is necessary to understand what pre-conditions within this particular issue domain facilitate the systematic processing of information; and which heuristics activate elaborative processing. This is a topic worthy of further research.

2.6 Trust as an Information Processing Heuristic

One way to reduce the amount of effort applied to information processing associated with information about a particular topic is through adoption of the opinion of another person or group of people regarding a particular subject or

topic. This may represent an efficient strategy if an individual believes that the other person has systematically appraised the different attributes of a given topic, and, at the same time, possesses the expertise needed to judge the merits or otherwise of the information under consideration. Thus the extent to which a source is perceived to possess expertise may act as a cue that increases the likelihood of persuasion occurring, regardless of underlying arguments contained in the information *per se*.[65] Perceived honesty and lack of vested interest associated with promoting a particular view may also contribute to persuasion,[66] although honesty without expertise may not have value in this context.[67] If an individual trusts a particular information source, they may accept the conclusions of the information provided by the source, independent of the argumentation provided in support of those conclusions. An information source that is perceived to share the same values as the person receiving the message contained in the information is more likely to be trusted than a source where this value similarity is not perceived to exist. For example, information sources perceived to promote consumer or environmental protection are more likely to be perceived to be trustworthy than a source prioritising the economic interests of a private company or financial sector.

However, these effects may also be dependent of the type of hazard under consideration. For example, in the case of communication about microbial food safety, there is evidence that information source characteristics are less influential than message relevance in influencing risk perceptions associated with food poisoning, which is not the case with emerging food technologies.[68]

Prior attitudes, personal experience and even automated behavioural patterns such as habits may also serve as heuristic determinants of behaviour. In the case of routine tasks such as food preparation, one might expect that mental processes such as habits are very important.[56] Thus consumers may subconsciously 'reject' novel foods because they are not the products they normally purchase, despite the fact that they have no conscious objections to the products or the technology used to produce them.

A further potentially influential heuristic is the existing attitudes associated with food technologies, which consumers may utilise in order to simplify decisions associated with new technologies applied in the same production sector. Even for relatively new technologies such as nanotechnology, the influence of existing attitudes or new (persuasive) information about the technology in general is shown to be important,[69,70] as more negative (or indeed positive) attitudes are less likely to be amenable to change through presentation of contradictory but persuasive information.

2.7 Emotions, Risk and Attitude Change

Another heuristic process, the *affect heuristic*,[71] has been derived from the observation that risk and benefit perceptions are, in general, negatively correlated.[72] This emotion-related heuristic implies that when an individual is experiencing positive emotions about a specific activity or event, the risks

associated with the activity or event will be perceived as lower and the associated benefits as higher.[73] From this, it is predictable that that an individual experiencing a positive mood associated with a specific event or activity will shift towards a more positive attitude towards this event or activity if the information provided is also positive, whereas negative affect has the converse effect.[71] This also suggests that inducing specific emotions in the course of risk communication may facilitate successful processing of information. An individual's fear about the potential ill-effects associated with a specific event or behaviour has often been used as a 'cue' to motivate the consumer to process information systematically in order to achieve attitude changes,[74] although it should be noted that the results of research where fear has been applied as a potential motivator have been equivocal.[75] However, emotion may not necessarily result in elaborative processing of information. One of the problems with using emotional cues embedded in, or associated with, a persuasive message is the possibility that emotions in themselves represent heuristic cues, inducing heuristic information processing. If the motivational effect of a specific emotion is not aligned with the message itself, the effect of applying emotions may lead to unexpected effects.[76] For example, fear may primarily activate self-protective behaviour, regardless of the message that is being communicated. Thus if people are potentially frightened about the negative effects of an emerging technology, such as nanotechnology, then they will associate fear with any information provided about it. One consequence is that information about the benefits may be rejected or avoided by consumers, and avoidance may be independent of the contents of the information itself. The exact role of emotions in processing persuasive information is not well understood at the present time. Many researchers in communication science and social psychology focus on the distinction between positive and negative emotions. However, some researchers argue that emotions can have specialised functions.[77] For example, it is postulated that anger, or aggression, is an emotion that mobilises resources to confront and 'fight' a problematic situation, whilst temporarily minimising concerns about individual personal safety and short-term goals relating to self-protection.[43] It is arguable that, in certain situations, short-term disregard for self-protection may result in long-term benefits (e.g. by standing up against aggressors to show that you are not tolerant of the aggression expressed by them). The long-term positive effect, a lower probability of experiencing threat from aggressors, will compensate for the short-term negative effect of experiencing physical injury.[78] For example, in a recent study, it has been shown that when communication about preventing food-borne diseases was accompanied by relevant images designed to invoke disgust, participants changed their food preparation practices in such a way that safer food handling behaviours resulted. When the same messages were accompanied by aggressive images, the efficacy of the message was very much reduced, to the extent that it was even less effective than a version of the same message in which no emotional images were provided.[79] In this case, the emotion 'disgust' may be specifically associated with food, as it promotes a specific self-protective behaviour (non-consumption of the disgusting product[16]). It is not currently known if 'neophobia'

and perceptions of 'unnaturalness' associated with emerging technologies applied to food production evoke an emotion akin to 'disgust', which would result in consumer rejection of the associated products.

2.8 Balanced Information

Persuasive communication theories provide an infrastructure in which attitude change might be induced. However, consumer trust in the same information might best be developed by the provision of information about the risks, benefits and regulation of new products. Indeed there are ethical reasons to justify the provision of balanced, rather than persuasive, information about the potential risks and benefits of controversial emerging technologies.[80] The provision of balanced information implies that different points of view are presented, and that both risk and benefits are communicated, if these are relevant to the topic under consideration. However, mixed messages will always contain a partially counter-attitudinal message, which may differentially affect people with strong, weak, neutral or extreme prior attitudes. In the past, the solution to such unpredictability was to avoid presentation of mixed messages, which may not reflect the reality of the situation, or indeed be ethically valid. However, if an individual already has established attitudes about a particular topic, and the message, about either risks or benefits, does not align with the existing attitudes held by an individual, then the perceived bias of the communicator may result in increased distrust in the message source, whilst at the same time having no impact on an individual's attitudes regarding the same topic.[22] In any case, individuals have increasing access to different sources of information about a potentially controversial topic (for example, via the internet) and so are no longer reliant on the views of experts, in particular those whom they do not trust.[81]

2.9 Attitudinal Strength and Ambivalence

If an individual holds a strong attitude about a particular event or activity, the attitude is more likely remain to stable over time, and will be less susceptible to influence by persuasive information.[82] It is very difficult to change such a strongly held attitude though provision of counter-attitudinal information, although there is some evidence that, after repeated persuasion efforts, the attitude valence (*i.e.* whether the attitude is positive or negative) does not change, but the strength of the attitude may (slowly) decrease.[83] For example, at the time of writing, there is evidence to suggest that people hold a very weak attitude towards nanotechnology applied to food production, which is more amenable to influence by information persuasive of either the associated risks or benefits. In contrast, attitudes towards genetically modified foods are very negative and established, and relatively immune to the influence of additional information.[84] However, consumers may also be ambivalent about a particular technology and its products; in other words, they perceive both good and bad

properties to be associated with the technology and its applications. In general, very positive, or very negative attitudes will be low in ambivalence, whereas more neutral attitudes may be either high or low in terms of ambivalence. The provision of balanced information about an issue, where people are already ambivalent, may actually result in a reduction of ambivalence and establishment of 'stronger' attitudes, perhaps because the provision of information enables more concrete attitude formation. This has indeed been demonstrated for the application of nanotechnology to food production,[84] leading to the conclusion that consumers currently are likely to be influenced by information as it becomes available, but that, once these attitudes are established, they will be less amenable to influence by new information.

In summary, consumer risk/benefit perceptions and consumer responses to persuasive information about the merits or otherwise of the novel products of nanotechnology will be one of the most important factors influencing the commercialisation of nanotechnology in the agri-food (and potentially other) consumer sectors.

While (bio)nanotechnology applied within the agri-food sector is still a technology 'under development', there has already been an extensive comparison with other recent technological developments, in particular the introduction of genetically modified foods (see Chapter 3). The example of genetic modification has demonstrated the consequences of failing to take into account potential reactions to the development and commercialisation of new technologies. Although some lessons may have been learned from research into the societal responses to genetically modified foods,[85] consumer attitudes towards nanotechnology in general, and applied to food production in particular, may be influenced by the way in which the technology is introduced into society, as well as the effectiveness of the associated communication strategies. Research is urgently needed to further our understanding of the fundamental psychological mechanisms which determine individual responses to existing and emerging food issues, particularly where habit, emotion and information-processing heuristics may have an effect on consumer decision-making. Indeed, the traditional emphasis on risk communication (or communication about the absence of risk) may be less relevant to consumer decision-making, as it has become increasingly evident that consumers are making decisions about the acceptability or otherwise of specific foods and production technologies based on a complex interaction of perceptions of risk and benefit associated with specific food choices.

Recent theoretical advances in the area of social psychology are relevant to the development of effective risk/benefit communication strategies. Although trust has been extensively evaluated in this context,[21,69,70,85,86] we have reviewed some evidence to suggest that other heuristics may also be potentially influential determinants of consumer behaviour. These may include habit[71,87,88] and affect (or emotion).[89] Furthermore, the role of implicit memory may generate attitudinal associations which determine whether or not information results in attitude change,[91] and the differential impact of balanced information in cases of weak and strong attitudes needs to be better understood to predict

attitude changes.[84,90] In some situations, attitude activation (through inclusion of relevant cues in information) may be a more influential determinant of risk-related behaviour than providing additional formal knowledge about risk and safety.[91] The relative importance of these different factors in determining attitude change and their potential for interaction are not well understood and may vary across potential hazard type, indicating the need for development of case studies focusing on agri-food nanotechnology. In any case, it is inappropriate to assume that all consumers are homogenous with respect to their perceptions (whether related to trust or information needs), necessitating exploration of individual differences in this context. In particular, consumers may be differently motivated to search for information regarding risks and indeed benefits of emerging technologies.[87,92] Demographic and psychological factors may account for profound differences between different consumers regarding their responses to emerging technologies and their applications, as well as other risk issues. In particular, targeted information provision needs to be developed which meets the needs of different groups of consumers, as peoples' responses to risk/benefit information may also vary according to predictable individual differences.[93]

2.10 Conclusions

Food technology has evolved from being focused on the issues associated with food availability, to include, more latterly, additional foci on food safety, sustainability and functionality. Despite the intuitive appeal of all of these beneficial factors in providing the basis for consumer acceptance of emerging technologies applied to food production, consumer acceptance of the benefits of agri-food nanotechnology will not be 'automatic'. Understanding consumer psychology is essential if we are to understand and predict peoples' responses to (bio)nanotechnology in the agri-food sector. Developing a societally inclusive and theoretically based understanding of consumer responses to (bio)nanotechnology and its applications in the agri-food sector and beyond, represents an essential part of developing an effective research and development strategy, which will simultaneously address emerging societal needs and societal preferences for novel foods and production processes.

References

1. Anon, *Africa Res. Bull.*, 2008, **44**(11), 17622–17623.
2. Anon, *The Lancet*, 2008, **371**(9622), 1389.
3. S. H. Katz, *Anthropol. Today*, 2008, **24**(1), 1–3.
4. B. M. Popkin and S. Du, *J. Nutr.*, 2003, **133** (11), Suppl. 2.
5. K. H. Yoon, J. H. Lee, J. W. Kim, J. H. Cho, Y. H. Choi and S. H. Ko, *et al., The Lancet*, 2006, **368**(9548), 1681–1688.
6. J. Diamond, *Nature*, 2002, **418**(6898), 700–707.
7. G. C. Hillman and M. S. Davies, *J. World Prehistory*, 1990, **4**(2), 157–222.

8. J. Gimpel, *The Medieval Machine: The Industrial Revolution of the Middle Ages,* Pimlico, London, 1992.
9. K. Sale, *Rebels Against the Future: The Luddites and their War on the Industrial Revolution, Lessons for the Computer Age,* Perseus, New York, 1996.
10. R. E. Evenson and D. Gollin, *Science*, 2003, **300**(5620), 758–762.
11. R. Carson, *The Silent Spring,* Houghton Mifflin, Boston, MA, 1962.
12. J. H. Behrens, M. N. Barcellos, L. J. Frewer, T. P. Nunes and M. Landgraf, *Innovative Food Science and Emerging Technologies*, 2009, **10**(3), 383–389.
13. C. M. Bruhn, *J. Food Protect.*, 1995, **58**, 175–181.
14. L. J. Frewer, J. Lassen, B. Kettlitz, J. Scholderer, V. Beekman and K. G. Berdal, *Food Chem. Toxicol.*, 2004, **42**(7), 1181–1193.
15. R. Flynn and P. Bellaby, (Eds.), *Risk and the Public Acceptability of New Technologies,* Pelgrave, Hampshire, 2007.
16. D. A. Kessler, *Annu. Rev. Nutr.*, 1995, **15**, xiii–xxvi.
17. P. Rozin, in *Understanding Consumers of Food Products*, ed. L. J. Frewer and H. Van Trijp, Woodhead, Cambridge, 2007, pp. 3–29.
18. D. A. Kesseler, *Annu. Rev. Nutr.*, 1995, **15**, xiii.
19. W. Van Rijswijk, L. J. Frewer, D. Menozzi and G. Faioli, *Food Qual. Prefer.*, 2008, **19**(5), 452.
20. L. J. Frewer, C. Howard and R. Shepherd, *Sci. Technol. Human Values*, 1997, **22**(1), 98–124.
21. G. Hofstede and G. -J. Hofstede, *Cultures and Organizations,* McGraw-Hill, New York, 2005.
22. L. J. Frewer, J. Scholderer and L. Bredahl, *Risk Anal.*, 2003, **23**(6), 1117–1133.
23. M. F. Schenk, A. R. H. Fischer, L. J. Frewer, L. J. W. J. Gilissen, E. Jacobsen and M. J. M. Smulders, *Health Risk Soc.*, 2008, **10**(3), 263–282.
24. W. Verbeke, I. Sioen, Z. Pieniak, J. Van Camp and S. De Henauw, *Public Health Nutr.*, 2005, **8**(4), 422–429.
25. T. Bech-Larsen and K. G. Grunert, *Appetite*, 2003, **40**(1), 9–14.
26. B. Fischhoff, P. Slovic and S. Lichtenstein, *Policy Sci.*, 1978, **9**, 127–152.
27. P. Slovic, *Science*, 1987, **236**(4799), 280–285.
28. P. Slovic, *Risk Anal.*, 1993, **13**(6), 675–682.
29. P. Slovic, in *Theories of Risk*, ed. D. Goldring and S. Krimsky, Praeger, New York, USA, 1992, pp. 117–152.
30. P. Slovic, B. Fischhoff and S. Lichtenstein, *Risk Anal.*, 1982, **2**(2), 83–93.
31. L. Bredahl, *J. Consumer Policy*, 2001, **24**(1), 23.
32. S. Henson and J. Northen, *J. Agric. Econ.*, 2000, **51**(1), 90–105.
33. S. Miles and L. J. Frewer, *Food Qual. Prefer.*, 2001, **12**(1), 47–61.
34. R. Pardo, C. Midden and J. D. Miller, *J. Biotechnol.*, 2002, **98**(1), 9–24.
35. J. M. E. Pennings, B. Wansink and M. T. G. Meulenberg, *Int. J. Res. Market.*, 2002, **29**, 91–100.
36. M. Siegrist, *Risk Anal.*, 2000, **20**(2), 195–204.
37. P. Sparks and R. Shepherd, *Food Qual. Prefer.*, 1994, **5**(3), 185–194.
38. W. Verbeke, *Food Qual. Prefer.*, 2001, **12**(8), 489–498.

39. P. Pliner and K. Hobden, *Appetite*, 1992, **19**(2), 105.
40. H. Tuorila, L. Lähteenmäki, L. Pohjalainen and L. Lotti, *Food Qual. Prefer.*, 2001, **12**, 29–37.
41. P. Rozin and T. A. Vollmecke, *Annu. Rev. Nutr.*, 1986, **6**, 433–456.
42. P. Rozin, J. Haidt and C. R. McCauley, in *Handbook of Emotions*, ed. M. Lewis and J. M. Haviland-Jones, Guilford, New York, , 2000, pp. 637–653.
43. K. Oatley and P. N. Johnson-Laird, *Cognition Emotion*, 1987, **1**(1), 29–50.
44. K. R. Scherer, *J. Personality Social Psychol.*, 1997, **73**(5), 902–922.
45. L. J. Frewer and B. Salter, *Appl. Biotechnol. Food Sci. Policy*, 2003, **1**(4), 199–211.
46. C. F. Kreijl and A. G. A. C. Knaap, *Measuring Dutch Meals; Healthy Diet and Safe Food in the Netherlands*, RIVM, Bilthoven, 2004, No. RIVM-270555008.
47. H. A. Kuiper, H. P. J. M. Noteborn, E. J. Kok and G. A. Kleter, *Food Res. Int.*, 2002, **35**, 267–271.
48. M. C. Van Putten, L. J. Frewer, L. J. W. Gilissen, B. Gremmen, A. A. C. M. Peijnenburg and H. J. Wichers, *Trends Food Sci. Technol.*, 2006, **17**(6), 289–299.
49. K. F. Mulder, *Int. J. Environ. Sustainable Develop.*, 2005, **4**(1), 88–103.
50. M. H. P. Marvin, G. A. Kleter, L. J. Frewer, S. Cope, M. T. A. Wentholt and G. Rowe, *Food Control*, 2009, **20**(4), 345–356.
51. W. van den Hoogen, A. L. Meijnders and C. J. H. Midden, in *Jaarboek Sociale Psychologie*, ed. R. Holland, J. Ouwerkerk, C. van Laar, R. Ruiter and J. Ham, ASPO pers, Groningen, Nl, 2005, pp. 207–214 (in Dutch).
52. C. Fife-Schaw and G. Rowe, *J. Risk Res.*, 2000, **3**(2), 167–179.
53. S. Miles, O. Ueland and L. J. Frewer, *Br. Food J.*, 2004, **107**(4), 246–262.
54. N. D. Weinstein, *Science*, 1989, **246**(4935), 1232–1233.
55. L. J. Frewer, J. Scholderer and N. Lambert, *Br. Food J.*, 2003, **105**(10), 714–731.
56. A. R. H. Fischer and P. W. De Vries, *Health Risk Soc.*, 2008, **10**(4), 385–397.
57. S. M. Parry, S. Miles, A. Tridente and S. R. Palmer, *Risk Anal.*, 2004, **24**(1), 289–299.
58. J. Hansen, L. Holm, L. J. Frewer, P. Robinson and P. Sandoe, *Appetite*, 2003, **41**(2), 111–121.
59. R. E. Petty and J. T. Cacioppo, *Communication and Persuasion: Central and Peripheral Routes to Attitude Change,* Springer-Verlag, New York, 1986.
60. J. T. Cacioppo and R. E. Petty, *J. Personality Social Psychol.*, 1982, **42**(1), 116–131.
61. J. T. Cacioppo, R. E. Petty, J. A. Feinstein and W. B. G. Jarvis, *Psychol. Bull.*, 1996, **119** (2), 197–253.
62. R. E. Petty and D. T. Wegener, in *Dual Process Theories in Social Psychology*, ed. S. Chaiken and Y. Trope, Guilford Press, New York, 1999, pp. 37–72.
63. S. Chen and S. Chaiken, in *Dual Process Theories in Social Psychology,* ed. S. Chaiken and Y. Trope, Guilford Press, New York, 1999, pp. 73–96.

64. R. H. Fazio and T. Towles-Schwein, in *Dual Process Theories in Social Psychology*, ed. S. Chaiken and Y. Trope, Guilford Press, New York, 1999, pp. 97–116.
65. W. J. McGuire, in *The Handbook of Social Psychology*, ed. G. Lindzey and E. Aronson, Random House, New York, 1985, **vol. 2**, pp. 233–346.
66. L. J. Frewer, C. Howard, D. Hedderley and R. Shepherd, *Risk Anal.*, 1996, **16**(4), 473–486.
67. E. Van Kleef, J. R. Houghton, A. Krystallis, U. Pfenning, G. Rowe and H. Van Dijk, *et al., Risk Anal.*, 2007, **27**(6), 1565–1580.
68. L. J. Frewer, C. Howard, D. Hedderley and R. Shepherd, *Food Qual. Prefer.*, 1997, **8**(4), 271–280.
69. J. R. Eiser, S. Miles and L. J. Frewer, *J. Appl. Social Psychol.*, 2002, **32**(11), 2423–2433.
70. L. J. Frewer, C. Howard and R. Shepherd, *Agric. Human Values*, 1998, **15**(1), 15–30.
71. P. Slovic, M. L. Finucane, E. Peters and D. G. MacGregor, *Risk Anal.*, 2004, **24**(2), 311–322.
72. A. S. Alhakami and P. Slovic, *Risk Anal.*, 1994, **14**(6), 1085–1096.
73. M. L. Finucane, A. S. Alhakami, P. Slovic and S. M. Johnson, *J. Behav. Decision Making*, 2000, **13**(1), 1–17.
74. K. Witte and M. Allen, *Health Educ. Behav.*, 2000, **27**(5), 591–615.
75. R. A. C. Ruiter, C. Abraham and G. Kok, *Psychol. Health*, 2001, **16**(6), 613–630.
76. A. L. Meijnders, C. J. H. Midden and H. A. M. Wilke, *Risk Anal.*, 2001, **21**(5), 955–966.
77. J. S. Lerner and D. Keltner, *Cognition Emotion*, 2000, **14**(4), 473–493.
78. D. Evans, *Emotion: The Science of Sentiment,* Oxford University Press, Oxford, 2002.
79. M. J. Nauta, A. R. H. Fischer, E. D. Van Asselt, A. E. I. De Jong, L. J. Frewer and R. De Jonge, *Risk Anal.*, 2008, **28**(1), 179–192.
80. J. R. Houghton, G. Rowe, E. Van Kleef, L. J. Frewer, G. Chryssochoidis, O. Kehagaia *et al.*, *J. Food Policy*, **33**(1), 13–26.
81. L. J. Frewer and B. Salter, *Sci. Public Policy*, 2002, **29**, 137–145.
82. E. M. Pomerantz, S. Chaiken and R. S. Tordesillas, *J. Personality Social Psychol.*, 1995, **69**(3), 408–419.
83. Z. L. Tormala, J. J. Clarkson and R. E. Petty, *J. Personality Social Psychol.*, 2006, **91**(3), 423–435.
84. H. van Dijk, A. R. H. Fischer, J. De Jonge, G. Rowe and L. J. Frewer, The impact of balanced risk-benefit information and initial attitudes on post-information attitudes, submitted.
85. D. Parr, *Trends Biotechnol.*, 2005, **23**(8), 395–398.
86. G. Cvetkovich, M. Siegrist, R. Murray and S. Tragesser, *Risk Anal.*, 2002, **22**(2), 359–367.
87. W. Poortinga and N. F. Pidgeon, *Risk Anal.*, 2005, **25**(1), 199–209.
88. A. R. H. Fischer, L. J. Frewer and M. J. Nauta, *Risk Anal.*, 2006, **26**(5), 1323–1338.

89. B. Verplanken and S. Orbell, *J. Appl. Social Psychol.*, 2003, **33**(6), 1313–1330.
90. A. Spence and E. Townsend, *Appetite*, 2006, **46**(1), 67–74.
91. A. R. H. Fischer and L. J. Frewer, *Food Qual. Prefer.*, 2009, **20**(8), 576–585.
92. A. R. H. Fischer, A. E. I. De Jong, E. D. Van Asselt, R. De Jonge, L. J. Frewer and M. J. Nauta, *Risk Anal.*, 2007, **27**(4), 1065–1082.
93. M. Kornelis, J. De Jonge, L. J. Frewer and H. Dagevos, *Risk Anal.*, 2007, **27**(2), 327–335.
94. A. R. H. Fischer and L. J. Frewer, *J. Appl. Social Psychol.*, 2008, **38**(11), 2859–2884.

CHAPTER 3

Public Perceptions of Nanotechnologies: Lessons from GM Foods

DAVID BENNET

Kluyver Laboratory for Biotechnology, Delft University of Technology, The Netherlands; St Edmund's College, University of Cambridge; Secretary, European Federation of Biotechnology (EFB) Task Group on Public Perceptions of Biotechnology; Director, Cambridge Biomedical Consultants Ltd.

3.1 Background

Although very promising, nanotechnology products and applications for food may add new dimensions to many ethical, social and economic issues. There has been enormous investment globally in this field. Clear benefits are possible, including masking of taste and odours, protection of ingredients during processing, enhanced bioavailability and longer shelf-life of food. Examples are the nano-encapsulation of fish oils (omega-3 fatty acids) in breads and other foods masking the 'fishy' taste and improving shelf-life, 'intelligent' food packaging for the traceability of products and food preservation, nano-structured films and coatings strengthening bottles and other plastic wrapping material, and incorporation of nanosensors into food packaging material allowing detection of contaminants and pathogens in foods and their surrounding environment.

While some products are unlikely to be controversial, others raise concerns about human and environmental safety together with broader social and ethical

RSC Nanoscience & Nanotechnology No. 14
Nanotechnologies in Food
Edited by Qasim Chaudhry, Laurence Castle and Richard Watkins

Published by the Royal Society of Chemistry, www.rsc.org

issues. For the promises to be realised to achieve the maximum benefit of nanobiotechnological innovations in food for everyone, the way has to be paved for a safe, integrated and responsible approach to their introduction. This will also be a necessary condition for the sustainable competitiveness of research and development, and for the industries using them. Public engagement and dialogue are therefore of primary importance to raise informed awareness and reduce unreasoned or overreacted objection to nanobiotechnology, and to avoid unproductive and costly delays as has happened during the introduction of some other new technologies such as genetic modification (GM) in food and agriculture. Research has showed for over a decade now, and has continued to do so, that public attitudes to a technology such as GM and now nanotechnology are conditional on how it is used, the social distribution of benefits and risks, and the capacity of government regulation to respond to unforeseen future consequences.[1,2]

An absolute condition, *sine qua non*, to be able to benefit from the investments and the technology in general is its acceptance in the various fields of application and recognition of the benefit of the products by the general public or the anticipated consumer or user groups. The public must provide, in effect, a 'licence to produce' for the nano-enabled products before they can come to market. This is not only by endorsing the innovations *per se* but also by bringing in their 'tacit' knowledge and so suggesting products better designed for their needs or even providing ideas for novel applications. Indeed, research on the interactions between scientific and technological 'producers' and users and consumers has shown that increased interaction can provide for better innovations.[3] Taking the experiences of public engagement seriously may lead to significantly better decision-making. Public communication, engagement and dialogue are key in obtaining this 'licence to produce' and the benefits of such interaction.

3.2 Quantitative Public Opinion Surveys

Among the respondents to a Europe-wide survey in 2004 who were almost all involved in nanotechnology R&D or the related issues, there was a large consensus that nanotechnology will have a strong impact on European industry (90%) and on European citizens (80%) within 10 years.[4] However, knowledge about nanotechnology among the public in general is currently very low as was shown, for example, by the European Commission's Eurobarometer 64.3 survey: *Europeans and Biotechnology in 2005: Patterns and Trends*, during which 42% of the European public surveyed did not know whether nanotechnology 'will improve our way of life in the next 20 years', 'have no effect' or 'make things worse' (Figure 3.1).[5]

While still unfamiliar to many, more were optimistic about nanotechnology in 2005 than in 2002, the ratio of optimists to pessimists being eight to one. Nanotechnology is perceived as useful to society, morally acceptable and not risky. In comparison to people in the USA and Canada, Europeans see nanotechnology as more useful and have greater confidence in regulation.

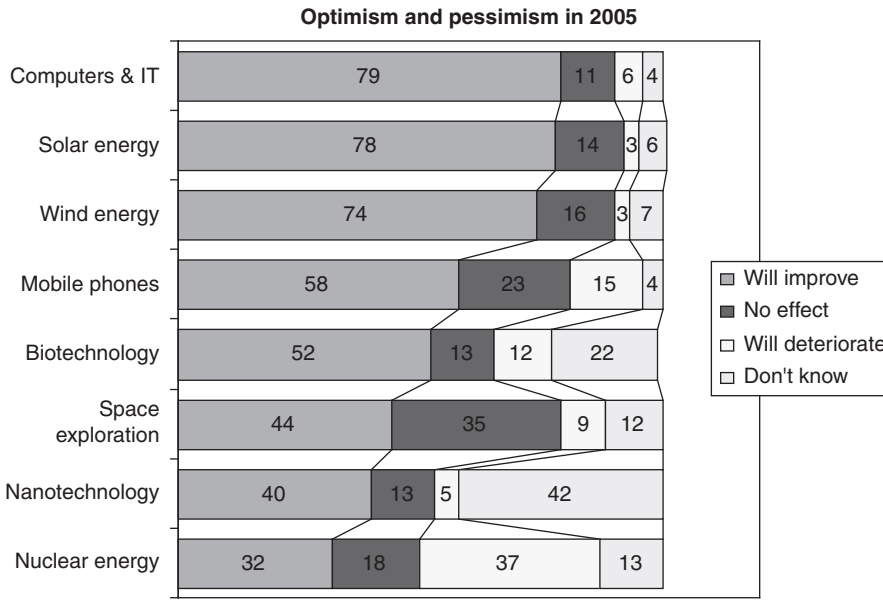

Figure 3.1 Eurobarometer 64.3: Europeans and biotechnology in 2005.

The Eurobarometer 64.3 also showed that public trust in university scientists carrying out research in biotechnology was second only to medical doctors, and public trust of scientists in industry was only a little lower, and both had increased between 2002 and the end of 2005 when the survey was carried out (Table 3.1).

Consumer organisations somewhat increased their already high level of trust. Trust in environmental groups campaigning against biotechnology declined markedly during the period. On the other hand, medical applications are usually generally welcomed and especially by patients' organisations who typically make great efforts on behalf of their members.

In November 2007 *Which?*, the leading UK consumer information and advocacy group, commissioned a survey among the general public, with 2091 adults interviewed, which also highlighted low levels of awareness for nanotechnologies and how they are being used. Six in ten adults had not heard of the term 'nanotechnology' (61%). Among those aware of the term, around two-thirds (64%) believed nanotechnologies were already being used to develop consumer products of some kind; just under one in twenty did not think they were being used to develop any (4%), but one-third simply did not know (33%).[6]

There is a similar low level of knowledge about nanotechnology in the USA where more than 70% responded 'neutral' to a survey question that asked participants to circle the word that represented their overall opinion of nanotechnology and its potential impact on their life and society.[7] It should be realised that all these indications of low levels of knowledge are quite possibly

Table 3.1 Eurobarometer 64.3: Europeans and biotechnology in 2005, trust in key actors and 1999–2005 trends

	% in 2005 Trust surplus/deficit (Base including 'don't know's)		*Trust surplus/deficit (Base excluding 'don't know's)*		
	Doing a good job	*Not doing a good job*	*1999*	*2002*	*2005*
Medical doctors keeping an eye on the health implications of biotechnology	75	8	72	80	79
University scientists doing research in biotechnology	**73**	**8**	-	**73**	**78**
Consumer organisations checking products of biotechnology	70	10	72	73	76
Scientists in industry doing research in biotechnology	**64**	**15**	-	**55**	**60**
Newspapers and magazines reporting on biotechnology	61	18	53	57	49
Farmers deciding which crops to grow	58	20	46	44	44
The European Union making laws on biotechnology for all EU countries	54	19	-	48	42
Industry developing new products with biotechnology	53	21	12	20	41
Television reporting on biotechnology	59	22	-	-	39
Environmental groups campaigning against biotechnology	**50**	**24**	**54**	**56**	**35**
Our government in making regulations on biotechnology	50	23	22	27	33
Shops making sure our food is safe	56	26	46	39	32

even overestimates because some people do not care to admit that they do not know about something when interviewed. Notwithstanding, however, the 2006 National Science Foundation (NSF)-funded survey in the USA of public perceptions of nanotechnology products found that US consumers are willing to use specific nano-containing products when the potential benefits are high, even if there are health and safety risks.[8] This low level of both public awareness and perceptions of risk continues, as is shown by the 2008 USA Woodrow Wilson report *Awareness of and Attitudes Towards Nanotechnology and Synthetic Biology*, with virtually unchanged findings from two years earlier.[9]

However, as a recent article in *Nature* stressed, the major new development in shaping public opinion is the importance of the internet:

> *. . . the Internet is overtaking television as the public's main source of science news. This means that a larger global audience can now access, on demand, a great diversity of science coverage from media outlets around the world. Moreover, the public are no longer just passive consumers of information. The Internet is now the first place people go to look for more information on a scientific topic, such as stem cells or climate change. Thanks to the Internet, in short, one could argue that the overall state of science communication is better now than at any time in the past.*[10]

This is borne out by the findings of the November-December 2007 Eurobarometer *Attitudes of European Citizens Towards the Environment* survey in which the internet had risen from 11% in 2004 to 24% as a main source of information on environmental issues for the European public.[11]

The very low awareness of nanotechnology by the public worldwide, and the very high percentage of 'don't knows' and 'neutrals' in the European and USA surveys compared with other technologies provides the opportunity for improving public understanding and initiating a balanced public dialogue from the outset. There is evidence of enthusiasm among the public for the anticipated social benefits of nanotechnologies such as generation of new medical innovations or sustainable technologies or benefit to the economy. The term 'nano' as a descriptive epithet currently has positive associations as evidenced by its use in marketing the 'iPod nano' and 'Tata Nano' (the new Indian car, currently costing €1570, which is expected to revolutionise the Indian car market).

On the other hand, there is the possibility that nanobiotechnology, through lack of awareness and knowledge about the advanced sciences and technologies involved, together with, importantly, their impacts, may become conflated into a single, undifferentiated, stereotypic image and label, whether positive or negative, in the public, media and political perceptions. Public engagement and dialogue, particularly via the internet, is absolutely imperative to raise informed awareness and prevent unreasoned or overreacted objection to nanotechnology and to avoid its possible erosion or loss by leaving a perception 'vacuum' to be occupied by campaigning environmental NGOs, as has happened during the introduction of some other new technologies such as genetically modified (GM) food and agriculture.

In nanotechnology in food, like GM, there are many different applications that lead to quite different public perceptions and risks by highlighting and assessing them with the widest possible range of inputs. In some cases, this may enhance the uptake of nanobiotechnology and in others may possibly reduce it but, unlike during the development of GM, this would result from a very well planned approach with the widest possible input and most informed and rational manner for handling the issues. As the authors of the NSF study concluded:

> *Transmitting the latest information about both risks and benefits, in a timely, thorough and transparent way, will minimise the likelihood of a polarised public debate that turns on rumour and supposition.*

3.3 Qualitative Public Opinion Research

A considerable number of qualitative research studies using focus group methods have been carried out on the wider public's attitudes to new technologies such as GM and nanotechnology. One of the earliest and largest was that on *Public Perceptions of Agricultural Biotechnologies in Europe*, conducted during 1998 to 2000 with 55 focus groups in the UK, Spain, Italy, France and Germany.[12] Others on nanotechnology have been organised more recently, such as the six initiatives in the UK reviewed by Nanotechnology Engagement Group.[2] The findings of these kinds of studies, while not statistically quantifiable like those of large population surveys (such as the Eurobarometer and others cited earlier), do provide much greater insight into the underlying values, motivations and desires involved.

Four common lessons emerge.

1. Public attitudes are formed not only in relation to particular technologies but also to the policies and values that influence the direction of technological development and to the social and political conditions in which they develop. People are not only concerned with the potential benefits and risks of nanotechnologies but also about whom the benefits and risks are most likely to affect.
2. Public attitudes to risk, uncertainty and regulation tend to be concerned with their views of the ability of regulations and regulatory authorities to manage complex risks. Although many concerns focus on potential risks such as toxicity of manufactured nanoparticles, members of the public seem as concerned with government's and industry's ability to deal with potential long-term risks and uncertainties associated with nanotechnologies as with the risks themselves. This includes a concern among some about the government's and industry's ability to ensure that potential benefits and risks are distributed fairly.
3. The issues raised by the members of the public mostly relate to broad aspirations and concerns about future implications of nanotechnologies,

rather than responses to particular technological developments. Even when more specific issues have been the focus of discussions, the final recommendations have tended to be broad in scope: addressing topics such as 'all manufactured nanoparticles' or 'companies using nanotechnologies in the environment'.

4. There is consistent demand for more open discussion and public involvement in policy making relating to science and technology overall than has been afforded up to now.

The fundamental questions in people's minds are:

- 'Whose agenda is it?'
- 'Whose interests are being served?' and
- 'Do I have choice and influence on the matter?'

The answer in the first two cases has to be 'everybody's' and in the third 'yes', and demonstrated to be so, directly in the resulting products and services, indirectly via jobs, taxes and the economy to living standards, and by engaging with and involving the public. Otherwise the likelihood is that the 'licence to produce' referred to at the outset is not granted or is withdrawn, and nanotechnology faces criticism and resistance.

3.4 Equivocal and Adverse Stances to Nano(bio)technology

Early in the development of nanobiotechnology, the Canada-based Action Group on Erosion, Technology and Concentration (*ETC* Group) published a report in January 2003 on the convergence between nanotechnology and biotechnology in general[13] and a second specifically on applications of nanotechnology in agriculture and food in November 2004.[14] One environmental NGO in particular, Greenpeace UK, also took an early interest in nanotechnology and in 2005 carried out *NanoJury UK,* which brought together 15 people from different backgrounds in the north-east of England in a dialogue intended to have an impact on policy.[15] It stated that:

> *Greenpeace does not have a stance on nanotechnology as a whole, because the applications will be too diverse, including information technology, pharmaceuticals, defence and energy. The first applications of nanotechnology look to be in making faster computers and helping doctors with better diagnostic tests.*
> *There may be some very beneficial uses of nanotechnology if it is directed towards, for example, a genuinely clean energy system with the prospect of very efficient lighting, cleaner manufacturing processes and cheap, efficient solar cells. However, these are some years from deployment, so whether they*

> *happen or not depends on a host of decisions about research and development funding now and over the next few years.*
> *Greenpeace has concerns about the use of 'nanoparticles' – very tiny particles which are so small that their chemical and biochemical properties will be different from the familiar bulk solids, and may be hazardous to human health and the environment. We want to see a moratorium on the release of nanoparticles to the environment until evidence that it is safe (for the environment and human health) is clear. In the longer term nanotechnology could produce self-replicating 'machines' whose proliferation could be environmentally problematic.*[16]

Friends of the Earth's nanotechnology project 'aims to catalyse debate on what is set to be one of the defining issues of our time'.[17] In May 2006 it published its report '*Nanomaterials, Sunscreens and Cosmetics: Small Ingredients Big Risks*'[18] commencing with:

> *In one of the most dramatic failures of regulation since the introduction of asbestos, corporations around the world are rapidly introducing thousands of tons of nanomaterials into the environment and into the faces and hands of millions of people, despite the growing body of evidence indicating that nanomaterials can be toxic to humans and the environment.*
> *Friends of the Earth believes that there are at least several hundred cosmetics, sunscreens and personal care products which contain engineered nanomaterials that are commercially available right now.*
> *Our research demonstrates that nanoparticles have entered just about every personal care product on the market, including deodorant, soap, toothpaste, shampoo, hair conditioner, sun screen, anti-wrinkle cream, moisturiser, foundation, face powder, lipstick, blush, eye shadow, nail polish, perfume and after-shave lotion.*

In a report published on 11 March 2008,[19] Friends of the Earth argued that it had identified at least 104 food and agricultural products either containing untested and potentially hazardous manufactured nanomaterials, or manufactured using nanotechnology, and that the real number of products is much higher 'given that many food manufacturers may be unwilling to advertise the nanomaterial content of their products'. It called on European policymakers to adopt comprehensive and precautionary legislation to manage the risks raised by the use of nanotechnology and also called for:

> *. . . a moratorium on the further commercial release of food products, food packaging, food contact materials and agrochemicals that contain manufactured nanomaterials until nanotechnology-specific regulation is introduced to protect the public, workers and the environment from their risks, and until the public is involved in decision making.*

As of January 2008 the Soil Association, an influential organic food movement organisation in the UK, with Prince Charles as its patron, banned the use of man-made nanomaterials from all its certified organic products, saying in its press release:

> *This applies particularly to health and beauty products, but also to food and textiles. Ahead of the Government, we are the first organisation in the world to take action against this hazardous, potentially toxic technology that poses a serious new threat to human health.*
> *Whilst the Soil Association recognises there may be benefits from nanotechnology – it has the potential to radically, and positively, transform many sectors of industry including medicine (e.g. delivering drugs that target specific cells) and for renewable energy such as fuel and solar power. Yet, of the $9 billion per year being invested globally in nanotechnology, much is going to the development of cosmetics and health products. Many well-known companies such as L'Oreal, Unilever, Boots and Lancome are already developing and introducing these super fine particles into their products and none of these products are required to have labelling to warn consumers.*[20]

Indicatively, the Soil Association's press release sets out its answers to the question:

> *Is nanotechnology like GM?*
> *There are many parallels with GM in the way nanotechnology is developing. As with GM:*

- Commercial opportunities have run ahead of scientific understanding and regulatory control. The risks of nanotechnology are still largely unknown, untested and unpredictable.
- The industry is trying to win over Government backing with compelling claims about the benefits of the technology and win over consumers by promoting individual products, whilst neglecting the fundamental issues of safety.
- Initial studies show some negative effects and there is a list of potential health impacts that have yet to be investigated by scientists.
- Regulators have not reacted to the scientific evidence of health effects for products that are already commercialised (titanium dioxide nanoparticles), instead accepting industry reassurances and unpublished industry evidence.
- The standard of proof is being set very high for any concerns, but low for reasons to dismiss concerns and without the context of a body of established scientific knowledge to judge conflicting arguments.
- Concerns are being downplayed on the basis of absence of any consensus over health problems and with arguments that some nanoparticles occur in nature or have been produced by industry for some time (true, but not

on the scale and with the chemical range being developed now; anyway, health concerns exist for some of these such as air pollution).

What is worse than GM is that there is no official assessment process or labelling of the products, and nano substances are being rapidly introduced to the market. This is a very bad starting point for the responsible introduction of a powerful new technology.

In a recent horizon-scanning exercise in the UK, 35 representatives from organisations involved in environmental policy, academia, scientific journalism and horizon scanning were asked to use wide consultation to identify the future novel or step changes in threats to, and opportunities for, biodiversity that might arise in the UK up to 2050, but that had not been important in the recent past. At least 452 people were consulted.[21] The *Journal of Ecology* article reporting the findings stated, *inter alia*, in relation to nanotechnology:

> *Benefits to biodiversity might arise from reductions in pollution and reduced bioavailability of chemicals following binding to nanoparticles. Challenges ahead include nanostructures, or their debris, that mimic functions found in cells (e.g. electron transport). Here, effects at surfaces or pores central to functioning in biological and environmental systems may be most important. If use becomes widespread or the structures are incorporated into 'near-living' systems, new approaches to risk will be needed.*

3.5 Public Consultation, Dialogue, Involvement, Engagement, *etc.*

It has now become very clear that knowledge and understanding of the science and technology behind new technologies is relatively less important in public perception and opinion forming. As the Eurobarometer 64.3 survey *Europeans and Biotechnology in 2005* showed, 90% of people had never attended a public meeting about biotechnology, only 2% saying 'frequently' and 3% 'occasionally', although these again may well be overestimates (Figure 3.2).

In this age of mass communications, people are overloaded with information and have many competing demands on their time and attention. What is of primary importance to the individual is the possible impact of a technology on their own life and on the lives of their close relatives and friends, and how they may be affected personally, both physically and emotionally. When there is a 'need to know', then there is attention, desire and seeking for information, and consideration of it. On the other hand, one of the lessons, hard-learned, from the GM debate is that the vast majority of European people are not really interested in science, do not understand it well and do not want to unless they have a personal need to. Otherwise their interest in science and technology is largely as spectacle, entertainment or controversy.

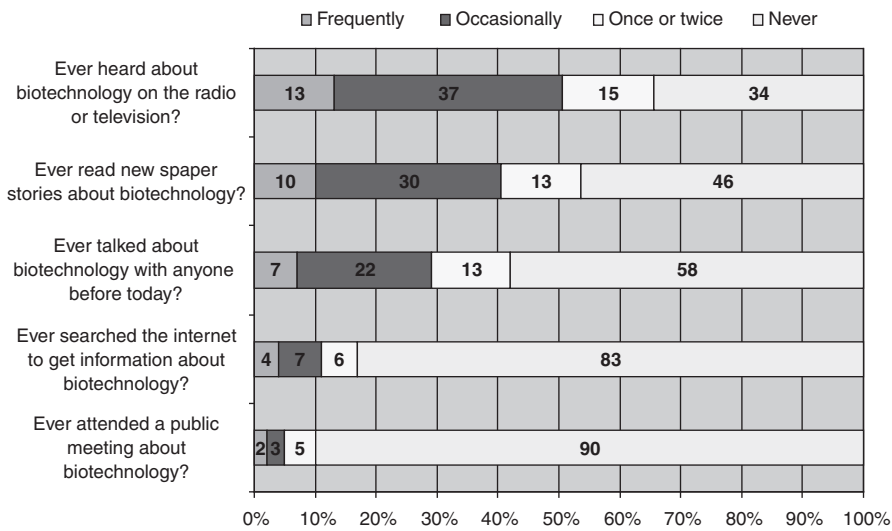

Figure 3.2 Eurobarometer 64.3: Europeans and biotechnology in 2005, participation in activities concerning biotechnology.

Similarly much experience now with various forms of public consultation, such as consensus conferences, citizen's juries, national debates, *etc*., leads to the conclusion that they serve to attract almost exclusively those with already committed views in relation to the topic and quite possibly polarise them further in their pre-determined opinions, negative or positive, as a result of the experience. Indeed, the final Government report of the UK countrywide *GM Nation?* dialogue carried out in 2003–4 which had a final budget of around £650,000 commented that, broadly speaking, it 'reflects the views of people who are regularly engaged in politics and current affairs. Such people are far more likely to be uncertain, suspicious or hostile towards GM and to have made up their minds about it.' and that 'Wait and see means wait rather than see'.[22]

3.6 Regulatory Issues

Nanomaterials, including their applications in nanomedicine, food products and their preparation, processing and storage, and environmental protection and remediation fall under the scope of existing health, safety and environmental regulation, even if they are not explicitly covered. However, implementation in many areas is difficult as current methodologies for identifying hazards and evaluating risks of substances may not allow the properties of substances in the nanoscale to be assessed. There is currently a lack of specific risk assessment procedures together with, until very recently, guidance and standards detailing how they are to be applied to nanomaterials. In the absence of specific guidance, researchers and companies working with nanomaterials

and regulatory authorities have used existing guidelines together with already available knowledge on the specific properties, processes and uses of nanomaterials in order to assess how to meet regulatory requirements and when it is necessary to intervene for health, safety or environmental reasons.

The European Group on Ethics (EGE) in its Opinion 21 on the ethical aspects of nanomedicine published in January 2007 did not propose any new regulatory structures specifically dealing with nanomedicine at this point, and argued that any changes should be made within existing structures with the focus being on the implementation of existing regulations.[23] This view is shared by the Working Group on Regulation of the ETP Nanomedicine, which is currently taking a closer look at the regulatory framework for medical devices and medical products.

The European Commission is carrying out a regulatory review as part of its Action Plan, which at the time of writing is to report in the near future. Its Interservice Group, without excluding regulatory change similarly with the EGE, considers it likely that it will not be the regulation itself that needs improving but the implementation of it. It is emphasised that there is a clear difference between a statement saying that uncertainties in knowledge mean it may be difficult to implement existing regulation and a statement saying that more regulation is needed.[24] There appears to be an almost general consensus at the present time that regulation needs to be both flexible and responsive to the findings of toxicology and risk assessment research. These issues are discussed in more detail elsewhere in this book (Chapter 10).

3.7 Possible Way Forward

Based on this experience, the answer would seem to be to concentrate on a range of public communication and engagement activities which have been found to have significant meaningful effect, and which are practicable within the given circumstances. They need to enable the views of all stakeholders, including, notably, those of consumers (which is all of us), to be brought together in considering the possible impacts of nanobiotechnology on everybody's lives.

Scientists involved in nanobiotechnologies need to be encouraged, practically supported and trained in engaging and communicating with the public, and in their awareness of ethical and societal issues. Most scientists see the need to communicate their work more widely, and some do it very well, but many are hesitant for reasons of priority of research, publication and career, lack of training and confidence, and lack of tangible rewards. It can also be argued that, as public scientists, they have a responsibility to explain their science and its significance for the general good since they are the experts on their science, as well as for their own good as scientists.

Scientists who do undertake and involve themselves in these kinds of activities often find themselves pleasantly surprised by the public's ability to understand and discuss nanotechnologies and by the good reception that they

receive. Conversely, members of the public often report that, while previously they had viewed scientists as being 'pompous' or 'arrogant', in actual fact the experience in these face-to-face situations was quite the opposite. Clearly stereotypes do exist, they are important in their outcomes and these types of public communication activity do help each to learn about the other as well as the science, and lessen the stereotypes.

There is now considerable practical experience of carrying out these activities with meaningful and effective impact, and their range is wide, for example:

- well-organised and well-publicised laboratory 'open days' for the general public with 'hands on' activities
- partnering with local schools and colleges whereby scientists visit to give talks and discussions and the students come to the laboratories for experimental demonstrations and discussions not possible elsewhere
- similar kinds of activities with local bodies such as chambers of commerce, trade unions, women's organisations
- building relations with local journalists and TV and radio presenters for coverage of scientific topics and so that they come to the scientists as trusted experts for advice and comment on current topical issues
- likewise building relations with local and national politicians so they approach scientists as trusted experts when there are science-based questions in the political agenda
- novel, innovative use of theatre, dance, music, art, *etc*., involving the science and technologies and, more recently, the internet with video, blogs, *etc*.

These kinds of activities of course need time and funding, and also needs commitment, talent and confidence. They certainly detract from time and attention which otherwise would be devoted to research, teaching, publication, grant seeking and all the other demands which scientists face.

Science's place in society has changed, and while society is dependent on science, so is science on society, and has to respond. Nanotechnology at the present time is very well placed to do so, both because of the currently low level of knowledge about it among the public and generally good acceptance, and because it can draw on the experience from such as the GM and nuclear energy public debates, but also that there are signs that it may well raise doubts and face challenge. In this regard, public engagement and communication is the key to the way forward.

References

1. R. Grove-White, P. Macnaghten, S. Mayer and B. Wynne, *Uncertain World: Genetically Modified Organisms, Food and Public Attitudes in Britain,* Centre for the Study of Environmental Change, Lancaster, 1997.
2. K. Gavelin, R. Wilson and R. Doubleday, *Democratic Technologies? The Final Report of the Nanotechnology Engagement Group (NEG)*, Involve,

London, 2007: see http://83.223.102.125/involvenew/mt/archives/blog_37/Democratic%20Technologies.pdf
3. R. Smits and P. den Hertog, *Int. J. Foresight Innovation Policy*, 2007, **3**(1), 28–52.
4. http://www.nanoforum.org/dateien/temp/nanosurvey6.pdf?21022007172412
5. http://www.ec.europa.eu/research/press/2006/pdf/pr1906_eb_64_3_final_report-may2006_en.pdf
6. http://www.which.co.uk/files/application/pdf/Which%20Nanotechnology%20Citizens%20Panel_Opinion%20Leader_Report%20Autumn%20071-445-133279.pdf
7. O. M. Castellini, et al., *J. Nanoparticle Res.*, 2006, **9**(2), 183–189.
8. S. C. Currall, *et al., Nature Nanotechnol.*, 2006, **1**(3), 153–155.
9. Peter D Hart Research Associates, Inc (2008), *Awareness of and Attitudes Towards Nanotechnology and Synthetic Biology*, Washington, DC: see http://www.nanotechproject.org/process/assets/files/7040/final-synbioreport.pdf
10. *Nature*, 2008, **452**, 387–388, (Editorial).
11. http://ec.europa.eu/public_opinion/archives/ebs/ebs_295_en.pdf
12. http://csec.lancs.ac.uk/pabe/docs/pabe_finalreport.pdf
13. The Big Down: see http://www.etcgroup.org/en/materials/publications.html?pub_id=171
14. Down on the Farm: see http://www.etcgroup.org/en/materials/publications.html?pub_id=80
15. http://www.greenpeace.org.uk/tags/nanojury
16. http://www.greenpeace.org.uk/about/nanotechnology
17. http://www.foe.org/camps/comm/nanotech/
18. http://www.foe.org/camps/comm/nanotech/nanocosmetics.pdf
19. http://www.foeeurope.org/activities/nanotechnology/Documents/Nano_food_report.pdf
20. http://www.soilassociation.org/web/sa/saweb.nsf/848d689047cb466780256a6b00298980/42308d944a3088a6802573d100351790!OpenDocument
21. http://www.blackwell-synergy.com/action/showPdf?submitPDF=Full+Text+PDF+%28159+KB%29&doi=10.1111%2Fj.1365-2664.2008.01474.x&cookieSet=1
22. http://www.gmnation.org.uk/ut_09/ut_9_6.htm and http://www.defra.gov.uk/environment/gm/crops/debate/pdf/gmdebate-lessons.pdf
23. http://ec.europa.eu/european_group_ethics/activities/docs/opinion_21_nano_en.pdf
24. http://www2.cst.gov.uk/cst/business/files/Nano_European_Commission.doc

CHAPTER 4

Natural Food Nanostructures

VICTOR J. MORRIS

Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA, UK

4.1 Introduction

Food is an example of soft matter; it is a highly complex biological structure.[1] Despite this complexity, it is still possible to understand the functionality of food materials, by studying the contributions to functionality made by the principal components.[2] The main constituents of foods are proteins, carbohydrates and fats, all of which can be considered as naturally occurring nanosubstances. Within raw materials, and processed foods, these constituents can self-assemble into higher-order structures, many of which are themselves examples of naturally occurring nanostructures.

Food molecules, and the structures they form, have been studied extensively by standard physical, chemical and colloidal science techniques for many years. Many of these techniques generate spatially averaged information on the conformation of the food molecules and the nature of local interactions between the molecules.

Microscopy provides a method for visualising the actual structure of foods. Electron microscopy has proved to be invaluable in visualising and understanding the complex biological structures present in foods and food raw materials. The need to image in a vacuum has led to the development of elegant preparative methods for preserving and visualising the natural structures present. Another major advance in understanding food structure has been the development of probe microscopes in the 1980s. These microscopes helped to spawn the

RSC Nanoscience & Nanotechnology No. 14
Nanotechnologies in Food
Edited by Qasim Chaudhry, Laurence Castle and Richard Watkins

Published by the Royal Society of Chemistry, www.rsc.org

scientific areas now known as nanoscience and nanotechnology, by allowing the imaging and manipulation of material structures close to the atomic and molecular level. Atomic force microscopy (AFM) has permitted the investigation of biological structures at the sub-molecular level under more natural conditions. AFM has also allowed the investigation and understanding of previously intractable problems in food science.[3] Such understanding provides a basis for selecting or manipulating the natural nanostructures formed by food molecules, but through rational rather than empirical selection of new raw materials. For the improvement and new design of food materials from conventional processing methods, nanoscience enables the improvement of natural nanostructures, through the use of standard and accepted selection and processing methods.

In this chapter the nature of certain natural food nano-sized substances and the natural nanostructures they form will be described. It will be shown, through particular representative examples, how AFM has provided new insights into food nanostructures, and how this information has been, and can be, used to improve the quality and nutritional value of foods. This chapter will also discuss the likely status of new foods based on such approaches, particularly in relation to the on-going debate on nanotechnology and food.

4.2 Naturally Occurring Food Nanosubstances and Nanostructures

The key molecular components of foods are carbohydrates, proteins and fats. The natural nanostructures formed by carbohydrates and proteins are probably of most significance in understanding how to manipulate the structure of foods in order to enhance quality and nutritional value.

4.2.1 Carbohydrates

The carbohydrates that are most responsible for structuring foods are polysaccharides. Polysaccharides are used as thickening and gelling agents in processed foods.[4,5] The building blocks of polysaccharides are sugars. Polysaccharides are generally high-molecular weight chain-like structures with diameters typically a few nanometers in size. Thus they can be considered as naturally occurring nanosubstances.

The chemical structure of polysaccharides depends on the source and method of extraction. Both factors are of importance in determining the functionality of the biopolymer and the quality of the products formed. Very few food polysaccharides are homopolymers. Because of their complex structures individual polysaccharides often have distinct functional properties. Various polysaccharides can be gelled in different ways to produce different structures. Through the appropriate selection of polysaccharides, and the correct choice of conditions, it is possible to produce gels which set at room temperature, set on cooling, or set on heating. The gels can be thermo-reversible, thermo-irreversible and either clear or opaque.[5]

Polysaccharides are also the main structural components in plant tissue and plant storage systems such as starch. Starch is probably the most complex polysaccharide used as a gelling and thickening agent in food.[4,6] The material is produced in plants as a complicated semi-crystalline granular structure. The production of starch-based foods usually results in a partial or complete disruption of the granular structure and then a period, during preparation or storage, when there is a partial recovery (retrogradation) of structure. It is this retrogradation process that ultimately determines the textural and nutritional value of the food product. Understanding the native granular structure offers a route to the design or rational selection of new starches with unique or desired functional properties. AFM has provided a way of obtaining new structural information that yields the basis for such an approach.

4.2.2 Proteins

Proteins are essentially high-molecular weight linear polymers composed of a chain of amino acids. The composition and the sequence of amino acids determine the local secondary structure within the protein and the overall tertiary structure of the protein. In aqueous solution, the folded structure of a protein tends to bury hydrophobic regions within the interior of the molecule and expose hydrophilic regions on the protein surface. Most of the food proteins obtained from plants, blood or milk are globular structures, although the caseins are present in milk as complex colloidal structures called casein micelles. The micelles can be disassociated to obtain the individual casein proteins. Proteins such as myosin and collagen, which play specialised structural roles in meat tissue, are rod-like structures. The globular proteins are generally tens of nanometers in size and the diameters of rod-like proteins are a few nanometers in size. These molecules are also an example of natural nanosubstances.

During the processing of foods the proteins can be induced to aggregate. In the bulk, the protein aggregation can lead to an increase in viscosity or, at higher concentrations, the formation of gels.[5,7] Aggregation and gelation is normally induced by heat treatment. This leads to a low degree of unfolding of the protein structure, allowing the proteins to bind together on aggregation. Gelation can lead to clear or opaque gels, depending on the conditions. Opaque gels are formed at high ionic strength or at pH values close to the isoelectric point of the protein. Neutral pH and low ionic strength favours transparent gels. Microscopy suggests that the opaque gels are formed by colloidal-sized 'precipitates' that associate further to form particulate gel structures. Electron microscopy of gel precursor or sections of protein gels show the formation of branched fibrous structures. Although the opaque gels will be colloidal structures, the clear gels are believed to contain fibrous structures, with diameters of the order of tens of nanometers in size and thus these gels, or the gel precursors, can be considered as self-assembled nanostructures.

Proteins are also used to stabilise foams and emulsions.[8] This behaviour is determined by the structures that are formed by the proteins at air–water and

oil–water interfaces. When proteins adsorb at an interface, some degree of partial unfolding of the protein structure will occur, with the exposure of hydrophobic regions at the non-aqueous air or oil phases. The extent to which the protein unfolds will depend on the nature of the non-aqueous phase, and also on the protein structure. Unfolding will also expose new structural features at the surface of the proteins that will allow them to interact and form networks. Studies have been made to monitor the nature and degree of unfolding of proteins at interfaces,[9] and attempts have been made to correlate the mechanical behaviour of interfacial protein films with the intrinsic stability of the proteins, at least for films formed at air–water interfaces.[10] The formation of an ordered monolayer network of proteins at an interface is an example of the formation of a two-dimensional self-assembled nanostructure. Conventional interfacial techniques, such as surface tension or surface rheology, provide spatially averaged data on the interfacial structure: the molecular structure at the interface has to be inferred from such studies. AFM provides a new method for directly visualising the interfacial structures at the molecular level.[11]

Another important group of surface-active molecule present in foods are surfactants. Surfactants are usually small 'tadpole-like' molecules composed of a hydrophilic head group and one or more hydrophobic tails. When these molecules adsorb at interfaces the tendency is for them to arrange themselves with their heads in the aqueous phase and their tails in the non-aqueous phase. The small surfactant molecules are able to diffuse and adsorb at interfaces much more rapidly than proteins. They are thus much more efficient at lowering the interfacial tension.

An interesting situation arises in foods when both surfactants and proteins are present, since they compete for occupancy of the interface.[11 13] It is known that surfactants are capable of disrupting and, at sufficiently high concentrations, displacing the proteins. Such effects are the cause of instability in protein-stabilised foams or emulsions. However, the deliberate generation of instability provides a basis for controlling the partial coalescence and texture of certain classes of fat-based emulsions and foams, such as whipped products and ice creams. Thus, understanding such competitive displacement processes is of both academic and commercial value.

The small surfactant molecules can pack efficiently at the interface and are more effective at lowering the surface free energy: thus they are able to displace individual proteins. However, at interfaces the proteins associate to form networks. This should severely inhibit the displacement of individual proteins. Thus the basis of the competitive displacement mechanism is by no means clear cut. The ability to visualise the interfacial networks by AFM allows the mechanisms of displacement to be studied, understood and controlled.[11 13]

4.2.3 Nanoscience Studies of Food Structure

In order to understand the value of using the tools of nanoscience to probe food structures, and to use such information to modify and design structure and

function, it is useful to consider specific examples where such an approach has led to new understanding and to the potential to design structures to enhance function. This section will focus on the use of AFM to investigate the structure of starch, the major food carbohydrate consumed by humans, and the interfacial structures formed in food foams and emulsions. Foams and emulsions are one of the main classes of processed foods and emulsions are a major source of fat in the diet.

4.2.3.1 Starch: a Partially Self-assembled Nanostructure?

Plants store energy in the form of starch. Starch is synthesised as partially crystalline, spheroidal, granular structures.[6] The size and shape of the granules depends on the plant species, and also on the location of the granules within the plant tissue. The microscopic observation of starch granules under crossed polarisers reveals a 'Maltese cross' pattern characteristic of aligned crystallites within the granule: the measured birefringence suggests that the c axis of the crystals is essentially aligned in a radial direction. The current view of starch granule structure (Figure 4.1) is based on the known chemical composition of the granule, the structures of the molecular species released when the granule structure is disrupted or gelatinised, the effect of acidic hydrolysis (lintnerisation) and enzymatic hydrolysis on granule structure, electron microscopy of granules and a modelling of small angle X-ray (SAXS) and small angle neutron scattering (SANS) data on starch granules.[14] A characteristic feature of starch granules is a banded structure termed growth rings. The growth ring structure of the granules has been attributed to the presence of crystalline and amorphous shells within the granule. The main evidence for the existence of these

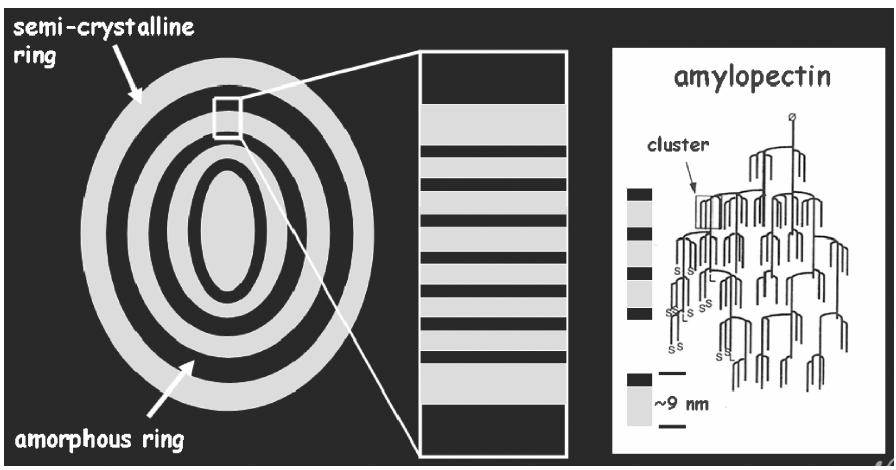

Figure 4.1 The currently accepted view of starch structure based on microscopy and SAXS data. An essential assumption of the model is the existence of sequential amorphous and crystalline growth rings.

shells is based on early microscopy studies of granules following relatively harsh acid hydrolysis or treatment with α-amylase. Such treatment led to the removal of alternate growth rings. Both treatments were shown to enhance the crystalline:amorphous ratio within the treated granules and hence were considered to preferentially hydrolyse amorphous regions of the granules.

Of the two distinct molecular species (amylose and amylopectin) that can be extracted from wild-type starches, the complex branched amylopectin molecules are considered to arise from the crystalline structures within the granule, whereas the essentially linear amylose is believed to arise from amorphous regions within the granule. The chemical structure of both amylose and amylopectin is based on a polymeric chain composed of α-(1→4)-D-glucose, with the small amylosic branches of the amylopectin linked at the 6-position of the glucose (α-(1→4,6)-D-glucose linkage points). The branches of the 'amylopectin' structure are present in the crystals as amylosic double helices, and the different packing of the helices throughout the granule determines the main A-, B- and C-type crystal forms of starch. Both high-resolution electron microscopy and interpretation of the SAXS data suggest that the crystallites are nanocrystals, typically ~9 nm in length. During biosynthesis of the granules, the length of the amylosic chains contained within the crystals is regulated. This determines the size of the crystals.

The currently accepted model[14] of starch granule structure (Figure 4.1) is based on the concept of alternate crystalline and amorphous growth rings. Whilst the SAXS models were being refined there were further developments in the electron microscopy of starch granules. Milder treatments were developed and employed to induce contrast within the granule. These largely microscopy based studies have led to an alternative model[15] of granular structure: the blocklet model (Figure 4.2). In this model there are no amorphous bands; merely alternating bands of different levels of crystallinity. A second refinement is the observed packaging of the nanocrystals into blocklets typically 50–100 nm in length: a higher order nanostructure within the granule. In the early schematic representations it was implied that the blocklet size was different in alternate bands.

AFM provides a new way to visualise the internal structure of starch granules.[16,17] The nature of the technique means that the granular structure can be viewed under 'near native' conditions. Thus, through the use of AFM it should be possible to compare and contrast the virtues of these two different models.

In order to visualise the internal structure of the granules it is necessary to cut open the granules. For isolated starch the granules need to be encased in a non-penetrating matrix:[16] early electron microscopy and AFM studies of starch granules embedded in resins that penetrated into the granules showed that the resin interfered with the visualisation of the 'native structure'. In imaging starch it is also possible[17] to make use of the natural self-embedding of starch in dry seeds and thus to image starch *in situ*. Contrast in the AFM images has been shown to arise due to the selective hydration of different regions within the granule:[16,17] this leads to swelling and softening of these areas and both these aspects contribute to the contrast in the AFM image. Thus controlled

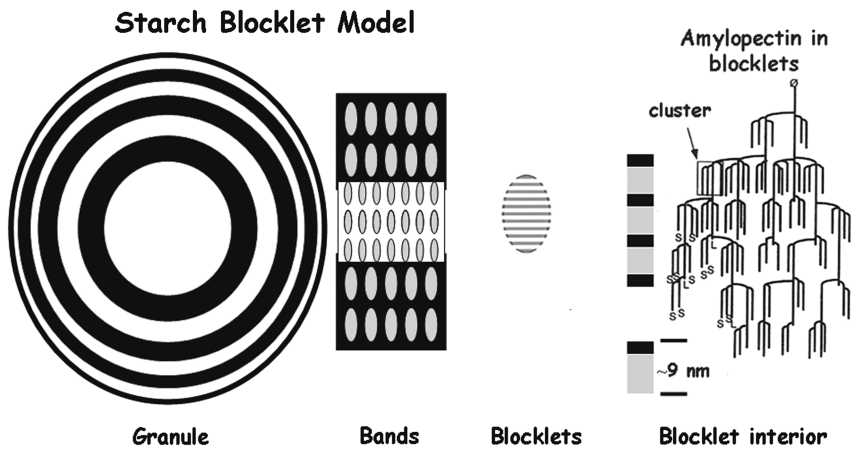

Figure 4.2 A schematic picture of the proposed blocklet model of starch granule structure. The granule contains hard and soft shells or bands. The bands are composed of blocklets in an amorphous matrix. The blocklets are laminated structures with the nanocrystals arising from the branched structure of the amylopectin.

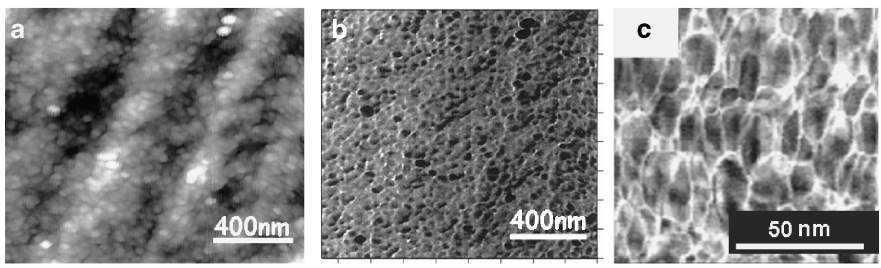

Figure 4.3 AFM images of the interior of pea starch granules. (a) Topographical images of the interior of smooth pea starch granules. The images show the blocklet structure within the bands. The bands are revealed by swelling of the matrix structure containing the blocklets. Alternate bands contain patches which show reduced swelling. (b) Phase images reveal the blocklet structure within these poorly swelling patches. (c) Force modulation AFM image of the interior of *r* mutant pea starch granules revealing a novel hard continuous network within the granule.

hydration of the cut faces of the encased starch is needed to optimise the images. The AFM images obtained are similar for different types of starches and are illustrated in Figure 4.3 for smooth pea starch imaged directly within the seed. The images confirm the blocklet structure of the granule. The blocklet structure is found to be continuous throughout the granule, without any evidence for amorphous bands. At lower resolution a banded structure is seen and

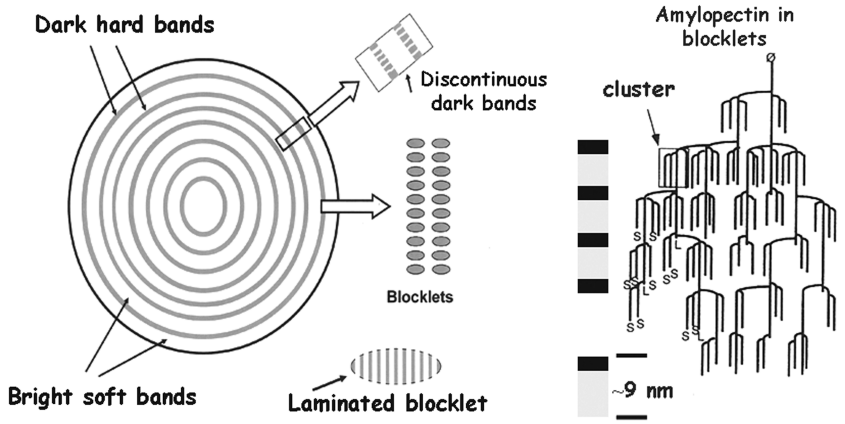

Figure 4.4 A schematic model of the starch granule structure derived from new AFM data. The granule contains alternating bands of different levels of crys tallinity attributed to variations in amylose concentration throughout the granule. The starch nanocrystals are packaged within blocklet structures.

this results from differential hydration of alternate bands, implying different crystalline/amorphous ratios in alternate bands. This effect has been attributed to variations in the amylose content throughout the granule. These observations have led to a revised model[17] of granule structure (Figure 4.4).

The regulation of the chain length of the branches of the 'amylopectin' determines not only the size, but also the melting temperature of the crystals: the melting temperature of amylose crystals increases with increasing chain length.[18] Heating of an aqueous suspension of starch granules results in 'gelatinisation' of the granules: the ordered structure within the granules breaks down, the granules swell, take in water and there is a release of the material termed amylose. Further disruption or dissolution of the residues of the granule yield the material called amylopectin. New insights have been obtained from the modelling of the SAXS data on the effects of water content on the crystallinity of the granules, and on the complex changes encompassing gelatinisation: in these models the advance has been the consideration of the branched amylopectin-type structures, contained within the granule, as side-chain liquid crystals.[19] However, despite the complexity of the gelatinisation process, it is clear that the chain length of the amylopectin branches dictates the gelatinisation temperature of the granule. Under normal processing conditions, in the presence of sufficient water, the elegant granular structure is lost.

In starch-based foods, both the amylose and amylopectin structures can recrystallise partially on cooling and storage.[20,21] The presence of such structures determines the digestion of starch-based foods.[22] Accessible, largely amorphous material can be broken down and appears as an elevated blood glucose level. The largely crystalline starch-based residues pass from the stomach and small intestine into the colon where they can be broken down by bacterial

enzymes: the breakdown of crystalline starch requires specialised enzymes that can recognise and bind to the crystal structures. The breakdown products are short chain fatty acids, chiefly butyrate, which are considered to contribute to protection against diseases such as colon cancer. Starch-based material which survives transit through the stomach and small intestine and is fermented in the colon is termed resistant starch (RS).[23] In attempting to prevent the occurrence of obesity, and the associated long-term conditions such as diabetes, strokes, heart disease and certain cancers, there is a drive to reduce the level and rate of release of glucose into the blood and to enhance the RS content of starch-based foods.

The ability to visualise the nanostructures within starch offers the prospect of designing, modifying or screening for starches with novel structures with enhanced functional and nutritional value. At present the RS content of starch-based foods is determined largely by processing. A target is to design or develop procedures for screening for or generating new starches with enhanced intrinsic RS values.

4.2.3.2 Interfacial Protein Networks: Self-assembly and Disassembly on Processing

AFM provides a means of visualising interfacial protein networks at the molecular level. Such information can be used to investigate the competitive displacement of proteins from interfaces by surfactants.[11 13] The interfacial structures can be studied by spreading or co-adsorbing the proteins at air–water or oil–water interfaces in a Langmuir trough and then, through the use of Langmuir-Blodgett methods, sampling the interfacial structure. AFM images of interfacial protein structures (Figure 4.5a) allow visualisation of individual proteins within the protein network. At this level of resolution an important observation is the presence of holes: heterogeneities or defects in the protein networks. The origin of such defects is not difficult to understand since, as the proteins adsorb, they can partially unfold and interact forming aggregates and then networks. The space available for further adsorption decreases, restricting unfolding, and interaction of newly adsorbed proteins. Eventually, the available holes can only accommodate passively adsorbed proteins, which would show little interaction with their neighbours, or the holes are too small to allow further protein adsorption. Such defects are vitally important in understanding competitive displacement by surfactants (Figure 4.5b).

AFM can be used to visualise the various stages of competitive displacement of interfacial protein networks by surfactants (Figures 4.5b and 4.6). The low-resolution images (Figure 4.6) show that the progressive colonisation of the interface by surfactant is heterogeneous in nature, rather than random homogeneous displacement of individual proteins. An important aspect of AFM images is that they generate a 3D representation of the interfacial structure and, from measurements of the area occupied by the protein, and the height of the protein network, it is possible to monitor the volume, or effective

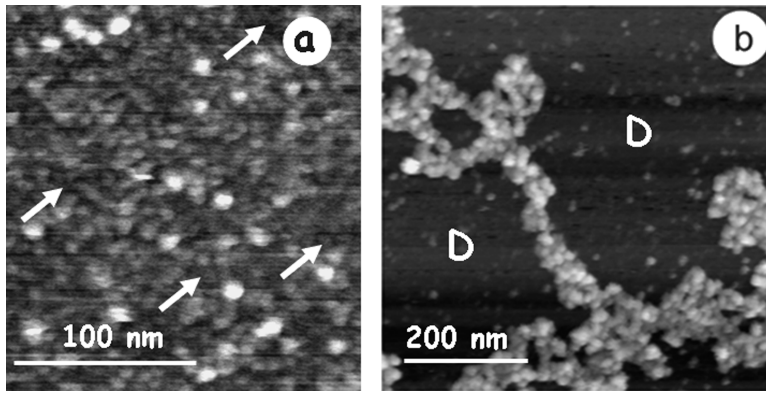

Figure 4.5 AFM images of interfacial protein networks. (a) A spread β lactoglobulin protein film at an air water interface. Individual proteins are visible and the network contains holes (some arrowed). (b) A protein (β lactoglobu lin) network close to displacement by growing surfactant (Tween 20) domains (D).

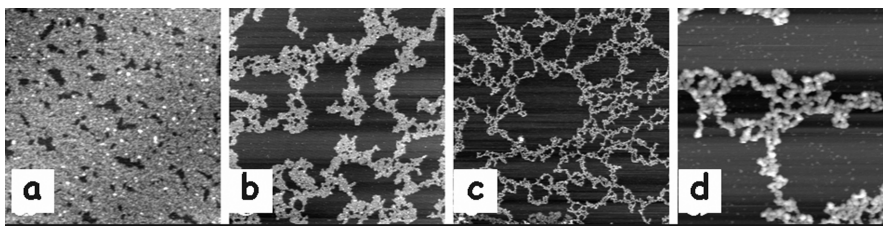

Figure 4.6 AFM images showing displacement of a spread protein (β lactoglobulin) network from an air water interface by a non ionic surfactant (Tween 20). Image sizes and surface pressures (Π) are (a) $1\times1\,\mu m$, $\Pi=18.6\,mN\,m^{-1}$, (b) $3.6\times3.6\,\mu m$, $\Pi=23\,mN\,m^{-1}$, (c) $5.2\times5.2\,\mu m$, $\Pi=29.1\,mN\,m^{-1}$, and (d) $1\times1\,\mu m$, $\Pi=29.1\,mN\,m^{-1}$. In the high resolution image (d) it is possible to see individual proteins in the interfacial network.

concentration of protein during the displacement process. For the data shown in Figure 4.6, the protein concentration remains constant until a critical surface pressure is reached where it quickly falls to zero (Figure 4.7). The key finding is that the protein is not released from the interface into the bulk until the protein network is broken. This then allows individual proteins, or protein aggregates, to be displaced by surfactant.

It is the defects in the protein network that allow displacement (Figure 4.8). They provide a weakness in the protein network that permits the surfactants to gain entry to the interface. These patches allow further adsorption of surfactant causing them to expand, compressing the protein network. As the area occupied by the surfactant increases, the protein are compressed and regions of the proteins are forced to fold and extend out into the bulk aqueous phase, whilst

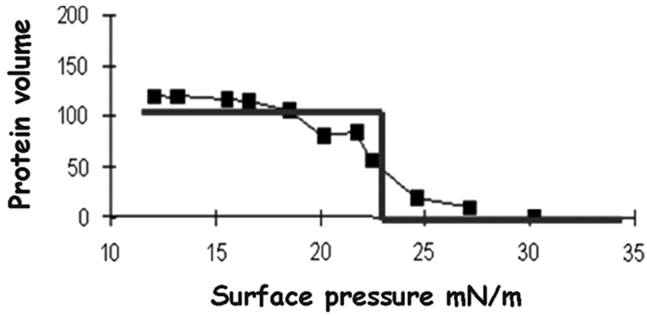

Figure 4.7 Change in protein volume with surface pressure during the displacement of protein (β lactoglobulin) from an air water interface by surfactant (Tween 20).

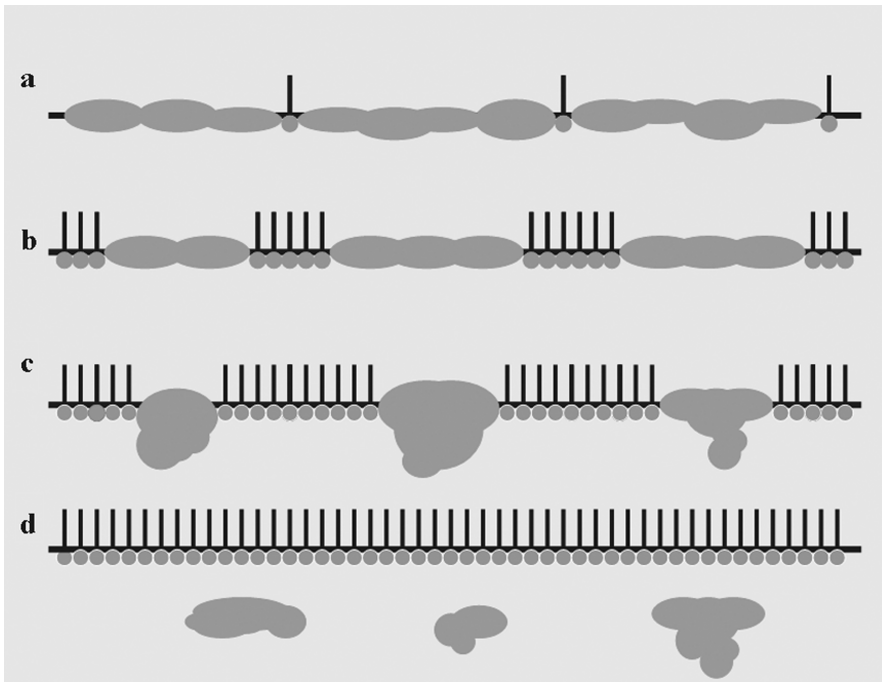

Figure 4.8 A schematic representation of orogenic displacement. (a) Surfactants have entered into defects and holes in the protein network at the interface. (b) These nucleated surfactant domains expand into larger domains compres sing the interconnected protein network and causing individual proteins to refold. (c) As the surfactant domains continue to grow they further compress the protein network. The network remains connected at the interface but it buckles and folds forcing regions of the proteins out into the aqueous phase. (d) Finally the protein network breaks and it becomes possible for individual proteins or protein aggregates to be expelled from the interface into the aqueous phase. The interface then becomes dominated by surfactant.

remaining interconnected at the interface. In the final stages of the displacement process (Figure 4.5b) the network becomes too stretched to survive further compression, and eventually breaks. Once the network is broken proteins can be completely expelled from the interface into the bulk.

The above mechanism, which has been termed 'orogenic displacement', is novel and was only deduced through the ability to image the interfaces at the molecular level.[11 13] Standard interfacial methods, that yield spatially averaged views of the interfacial structure, would not have identified the important role played by such structural defects in the network. The AFM data confirms the existence of elastic protein networks and demonstrates the new information that it is the mechanical strength of the network that is the crucial factor preventing displacement. Experiments have shown that this mechanism is generic[11 13] and applies to competitive displacement from both air–water and oil–water interfaces, for neutral and charged surfactants, and for both water-soluble and oil-soluble surfactants. By using AFM to probe interactions between deformable droplets in an aqueous medium, it has been also possible to demonstrate the orogenic displacement of elastic protein networks from the curved surfaces of finite sized oil droplets, confirming that the mechanism is applicable to real food emulsions.[24]

The studies on model, single protein systems have been extended to investigate and explain the displacement of mixed proteins.[11] This knowledge has been used to interpret the behaviour of complex protein isolates used as commercial foam stabilisers and emulsifiers.[25] The generic nature of the mechanism means that it is applicable to all food foams and emulsions. Because the orogenic mechanism is generic, the methods for inhibiting displacement are also generic; the only practical solutions are to strengthen the protein network or to prevent the surfactant reaching the interface.

The studies on interfaces illustrate how nanoscience provides new understanding and suggests new approaches to controlling food structure and function.

4.3 Designing Food Nanostructures

The idea of designing food nanostructures is to use the understanding gained through nanoscience to identify novel raw materials or to develop new food structures rationally, with desired functionality.

4.3.1 Designer Starches

A target for designer starches would be to develop or identify starches with enhanced RS values. If the gelatinisation temperature of starch could be increased then this could be used to restrict gelatinisation and enhance the RS content of the starch. It might be possible to produce *in planta* sources of pure RS or to generate populations of granules with different levels of intrinsic RS.

One way to alter the properties of starch is to manipulate the biosynthesis. Fortunately there is a range of natural biosynthetic mutants for most species of starch and new types of mutants can be produced by genetic engineering. The most common mutations are those that lead to variations in the amylose/amylopectin ratio. Changes in amylose content of the granule are generally considered to vary the relative proportions of the 'amylose-like' and 'amylopectin-like' structures in native starches. In practice such modifications can often lead to completely new physical structures for the granule. Some such modifications have been found to drastically alter the gelatinisation behaviour of starch granules. By visualising the changes in granule structure that give rise to changes in gelatinisation it should be possible to learn how to identify useful starches, or to define and explain the genetic origins of the changes in functionality.

This type of approach is best illustrated for studies on pea starches. Through studies on isogenic pea starches it has been possible to show that biosynthetic mutations that increase the amylose content of the granule also cause drastic changes in granule structure.[26 28] In particular the *r* mutant,[26] a mutation that reduces the activity of a starch-branching enzyme responsible for the assembly of amylopectin-type structures within the granule, results in a reduction of amylopectin content by $\sim 50\%$, but no significant changes in total crystallinity of the granules, although the crystal type changes from C-type to B-type. The mutation also leads to the presence of cracked and fissured granules which lack growth rings, show altered gelatinisation behaviour and do not gelatinise at temperatures $< 120\,^{\circ}C$. Lintnerisation studies have revealed that the crystalline regions within the granules contain a spectrum of amylosic chain lengths extending to higher molecular weights than those normally observed for the branches of native amylopectin molecules. Thus the crystal structure within the granule has changed and the spread in amylosic chain lengths would provide an explanation for the broadened gelatinisation behaviour. The use of AFM to image the internal structure of the starches reveals the origin of these changes.

The internal structure of the native granules is populated by semi-crystalline blocklets (Figure 4.3a and 4.3b) embedded in what is considered to be an amylose matrix (Figure 4.4). Light microscopy of the isolated starches from the *r* mutant[27] appear to show a mixture of normal granules, plus a population of abnormal, cracked and fissured granules. AFM images of these abnormal *r* mutant starches[27] suggest that they lack visible growth rings. High-resolution AFM images, designed to emphasis the harder, crystalline regions within the granules, seem to suggest the presence of a novel crystalline network, distributed throughout the granules (Figure 4.3c).[27,28] It appears that the interruption of the normal biosynthesis process leads to a de-regulation of the amylosic chain lengths which crystallise within the granule. The presence of normal and abnormal populations of starches would account for the spread of gelatinisation temperatures. The new networks formed in the abnormal granules would account for the cracking and fissuring of the granules since the brittle network would fracture on swelling and de-swelling of the granules during maturation within the seed or on extraction of the starch. The presence

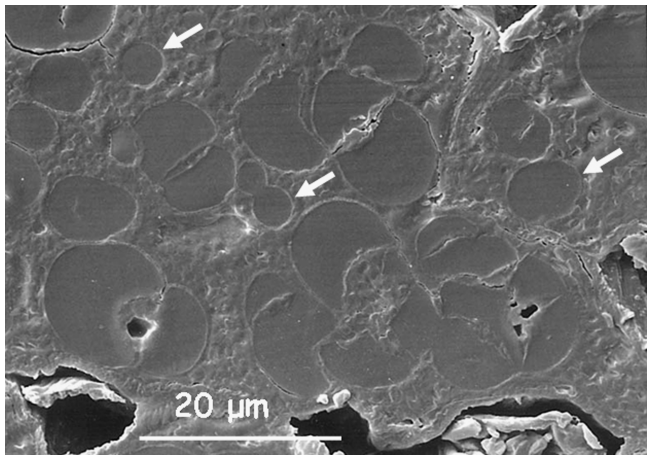

Figure 4.9 Scanning electron micrograph of a cut face of a *r* mutant pea starch seed (Greenshaft) showing the presence of normal immature starch granules (some arrowed) and abnormal mature cracked and fissured granules. (Unpublished data obtained by Dr M. L. Parker and Miss K. Gotts.)

of such a crystalline network would also inhibit swelling of the exposed face of the granule making it difficult to visualise any growth ring structure present in the granule.

Using the methods developed to allow for *in situ* imaging of the internal structure of starch granules within seeds[17] it has been shown (Figure 4.9) that the seeds of commercial wrinkled peas (natural *r* mutants) such as Greenshaft contain a mixture of immature granules that gelatinise normally, and mature granules, which are cracked and fissured, and do not gelatinise at less than 140 °C. Thus these seeds contain a blend of normal and intrinsically 'resistant starches'. Such commercial varieties provide a basis for examining the nutritional consequences of adding isolated blends of normal and RS granules to foods, or of the consequences on the diet of consuming foods which naturally contain blends of normal and RS starch.

The present studies suggest that mutations in starch-branching enzymes can lead to the production of starches with enhanced RS content. The potential cracking and fissuring of the granules suggests a simple phenotype for identifying the presence of unregulated crystalline networks within the granules.

4.3.2 Designer (Nano)foams and Emulsions

An understanding of the nature of protein layers at interfaces provides a basis for manipulating or designing interfacial structures to enhance food quality. Targets are enhanced shelf-life, improved sensory properties and enhanced nutritional value.

One approach is to consider strengthening the protein network, which should inhibit surfactant-based displacement, and improve shelf-life. Strengthening the protein network will also impact on the sensory aspects of food emulsions. Contrary to presently accepted beliefs, strengthening the protein network should enhance the texture and sensory appeal of food emulsions. The deformability of oil droplets is normally considered to be regulated by the interfacial tension, through changing the Laplace pressure within the droplets. Protein adsorption should lower the interfacial tension and make the droplets more deformable. In practice, direct measurements of droplet deformation[24] show that the adsorption of proteins and the maturation of the protein network enhance the stiffness of the droplets: it is the protein network elasticity that determines deformation. Studies of the small deformation behaviour of oil droplets in simple shear have also shown how elastic interfacial protein networks reduce deformability.[29] Less deformable droplets will show higher bulk viscosity and this will enhance texture and mouth-feel. Comparative studies of otherwise identical emulsions with 'elastic' rather than 'viscous' interfacial structures do show enhanced sensory attributes.[30]

Because the interfacial structures are self-assembling it is possible to manipulate the systems by changing the composition or the detailed processing of the food product. The quality of the product can be improved by conventional methods but in a rational rather than an empirical manner. New solutions are possible that would generate novel foods. For example, the protein network could be strengthened through cross-linking using chemical, enzymatic or physical treatments. Selective and sequential deposition of different molecular layers could also be used to strengthen or functionalise the interface: this is a form of nanotechnology but, since the layers self-assemble, the depositions could be achieved through relatively simple conventional chemical processing steps. Nanoscience offers novel solutions that could be applied to develop types of food with desirable textural or nutritional properties.

As well as trying to enhance the shelf-life and appeal of processed foods it is also possible to consider how deliberate manipulation of interfacial structures might be used to manipulate or control the digestion of fats. At present we know very little about the effects of digestion conditions on interfacial structures and their impact on the digestion of food foams and emulsions. During digestion, interfacial protein layers will be subjected to acid conditions in the stomach, the action of proteolytic enzymes in the stomach and small intestine, and exposure to bio-surfactants such as phospholipids and bile salts. In the small intestine the colonisation of the interfaces by bile salts is crucial to the surface location of lipase–co-lipase complexes, and the digestion of fats. Recent studies[31] suggest that bile salts will displace protein networks through an orogenic process. Thus manipulation of the strength of an interfacial protein network may offer routes to interfacial layers designed to moderate fat digestion. Clearly the strengthening of protein networks to an extent which completely eliminates any displacement by bio-surfactants would have undesirable effects. However, moderation of the extent of bile displacement could, through limiting the interfacial area occupied by bile salts, provide a means of

controlling the rate of lipase action and producing hormonal responses that induce satiety or loss of appetite. It is known that the longer fat remains in the intestine the more satiety is promoted: fat-induced satiety is intestinally mediated.[32] Once again nanoscience suggests new opportunities to manipulate food nanostructures but the processes used to generate such structures would be conventional.

4.4 The Status of Natural Nanostructures in Food

In the context of the current debate on the use of nanotechnologies in food, it is useful to consider the status of natural nanosubstances and nanostructures that are present, or may be formed during processing, in foods. Much of the debate is concerned with the accidental or deliberate inclusion of engineered nanoparticles (ENPs) in foods, and towards the steps needed to monitor, regulate and approve the use of nanotechnology derived food products, and possible labelling to inform consumer choice.

For the ENPs, formed from materials that are not metabolised by the body, there is at present insufficient information on the extent and mode(s) of uptake, the level and location of the accumulated material within the body and potential safety of such materials. An example here might be nano-silver, which is available as a food supplement, but for which there is little published data on the consequences of ingestion. Since nano-silver is marketed as an antimicrobial agent, amongst other safety aspects, the action of ingested nano-silver on beneficial microflora in the mouth or gut needs to be investigated. Here, there is clearly a need for additional data. Once such information becomes publicly available, then the acceptable daily intake (ADI) value for nano-silver may be found to differ from that of other physicochemical forms of silver. This may in turn require an indication of the use of nano-silver in foods through appropriate labelling. This is an example where there is a clear intention for the use of nanotechnology to engineer a new functionality into a food additive or food ingredient, and where there does seem to be a need for some form of labelling. There have also been claims that it is not possible to label foods to indicate the use of nanotechnology because all foods contain natural nanosubstances and nanostructures. However, there must be a clear distinction between the naturally occurring nanosubstances, such as proteins, which have been present in, and safely consumed in foods for a considerable period of time, and the insoluble/indigestible ENPs that may be added to a food product for certain new and novel properties.

For naturally occurring nanosubstances, such as proteins or polysaccharides, there are tried and tested procedures for regulation and use. The genetic engineering of proteins to reduce allergenicity, to enhance stability or enzyme activity, or the development and use of chimeric structures with enhanced functionality might be conceived as a form of nanotechnology, since it uses manipulation to generate designed structures. Such materials would require clearance as novel additives and ingredients and may even require labelling as

genetically engineered products. However, it is unlikely that they would require identification as the products of nanotechnology. The important point here is that these are modified materials, and there is a strong consumer lobby to ensure that the consumers can elect either to use or reject such products. However, where conventional processing techniques have been used to change the functionality of foods then this type of approach would be considered to be natural. Thus it is not unreasonable to assume that the use of nanoscience to enable the rational selection or processing of naturally occurring food materials may also be considered natural. The products of such approaches are likely to be novel foods, additives or ingredients, which may require clearance, but it is unlikely that the consumer would consider such products to be the result of nanotechnology *per se*.

4.5 Conclusions

Natural food constituents such as proteins and polysaccharides that generate structure and texture in food products are examples of naturally occurring nanosubstances. The processing of raw materials and ingredients into foods involves the breakdown and re-assembly of natural nanostructures. Nanoscience provides new opportunities to visualise and understand the behaviour of such structures. This information permits rational, rather than empirical, manipulation of food structures. Nanoscience also enables conventional and acceptable methods to be used for the selection and processing of naturally occurring nanostructures to optimise food quality. The nanostructures resulting from such processes, however, need to be clearly distinguished from the insoluble/indigestible ENPs that may be added to food products to achieve a certain taste, texture or functionality. Although the products of such approaches are still likely to require clearance as novel foods, additives or ingredients, it seems unlikely that they would be considered to be the 'products of nanotechnology' *per se*, or that they would need to be labelled as 'nanofoods'.

References

1. R. Mezzanga, F. Schurtenberger, A. Burbidge and M. Michal, *Nature Materials*, 1995, **4**, 729.
2. E. Dickinson and D. J. McClements, *Advances in Food Colloids,* Blackie, Glasgow, 1995.
3. V. J. Morris, in *Understanding and Controlling the Microstructure of Complex Foods,* ed. D. J. McClements, CRC Press Woodhead Publishing Ltd, Cambridge, 2007, ch. 8, p. 209.
4. V. J. Morris, in *Understanding and Controlling the Microstructure of Complex Foods,* ed. D. J. McClements, CRC Press, Woodhead Publishing Ltd, Cambridge, 2007, ch.1, p. 3.

5. V. J. Morris, in *The Chemical Physics of Food,* ed. P. S. Belton, Blackwell Publishing, Oxford, 2007, ch. 6, p. 151.
6. V. J. Morris, in *Functional Properties of Food Macromolecules,* ed. S. E. Hill, D. A. Ledward and J. R. Mitchell, Chapman & Hall, Aspen Publishers, Gaitherburg, 1998, ch. 4, p. 143.
7. A. H. Clark, in *Functional Properties of Food Macromolecules,* ed. S. E. Hill, D. A. Ledward and J. R. Mitchell, Chapman & Hall, Aspen Publishers, Gaitherburg, 1998, ch. 3, p. 77.
8. H. H. J. de Jongh, in *Understanding and Controlling the Microstructure of Complex Foods,* ed. D. J. McClements, CRC Press, Woodhead Publishing Ltd, Cambridge, 2007, ch. 2, p. 40.
9. A. H. Martin, M. B. J. Meinders, M. a. Bos, M. A. C. Stuart and T. van Vliet, *Langmuir*, 2003, **19**, 2922.
10. A. H. Martin, M. B. J. Meinders, M. a. Bos, M. A. C. Stuart and T. van Vliet, *Langmuir*, 2005, **21**, 4083.
11. V. J. Morris and A. P. Gunning, *Soft Matter*, 2008, **4**, 943.
12. A. R. Mackie, A. P. Gunning, P. J. Wilde and V. J. Morris, *Langmuir*, 1999, **210**, 157.
13. P. J. Wilde, A. R. Mackie, F. H. Husband, A. P. Gunning and V. J. Morris, *Adv. Colloid Interface Sci*., 2004, **108–9**, 63.
14. A. M. Donald, K. L. Kato, P. A. Perry and T. A. Waigh, *Starch*, 2001, **53**, 504.
15. D. J. Gallant, B. Bouchet and P. M. Baldwin, *Carbohydr. Polym*., 1997, **32**, 177.
16. M. J. Ridout, M. L. Parker, C. L. Hedley, T. Y. Bogracheva and V. J. Morris, *Biomacromolecules*, 2004, **5**, 1519.
17. M. L. Parker, A. R. Kirby and V. J. Morris, *Food Biophys*., 2008, **3**, 66.
18. G. K. Moates, T. R. Noel, R. Parker and S. G. Ring, *Carbohydr. Res*., 1997, **298**, 327.
19. T. A. Waigh, K. L. Kato, A. M. Donald, M. J. Gidley, C. J. Clarke and C. Riekel, *Starch*, 2000, **52**, 450.
20. M. J. Miles, V. J. Morris, P. D. Orford and S. G. Ring, *Carbohydr. Res*., 1985, **135**, 271.
21. M. J. Miles, V. J. Morris and S. G. Ring, *Carbohydr. Res*., 1985, **135**, 257.
22. P. Cairns, V. J. Morris, R. L. Botham and S. G. Ring, *J. Cereal Sci*., 1996, **23**, 265.
23. S. A. Bingham, *Proc. Nutr. Soc*., 1990, **49**, 153.
24. A. P. Gunning, A. R. Mackie, P. J. Wilde and V. J. Morris, *Langmuir*, 2004, **20**, 116.
25. N. C. Woodward, P. J. Wilde, A. R. Mackie, A. P. Gunning, P. A. Gunning and V. J. Morris, *J. Agric. Food Chem*., 2994, **52**, 1287.
26. T. Y. Bogracheva, P. Cairns, T. Noel, S. Hulleman, T. L. Wang, V. J. Morris, S. G. Ring and C. L. Hedley, *Carbohydr. Polym*., 1999, **39**, 303.
27. M. J. Ridout, M. L. Parker, C. L. Hedley, T. Y. Bogracheva and V. J. Morris, *Carbohydr. Res*., 2003, **338**, 2135.

28. M. J. Ridout, M. L. Parker, C. L. Hedley, T. Y. Bogracheva and V. J. Morris, *Biomacromolecules*, 2004, **5**, 1519.
29. P. Erni, P. Fiischer and E. J. Windhab, *Appl. Phys. Lett.*, 2005, **87**, 244104.
30. P. B. Moore, K. Langley, P. J. Wilde, A. Fillary-Travis and D. Melia, *J. Sci. Food Agric.*, 1998, **76**, 469.
31. J. Maldonado-Valderrama, N. C. Woodward, A. P. Gunning, M. J. Ridout, F. A. Husband, A. R. Mackie, V. J. Morris and P. J. Wilde, *Langmuir*, 2008 (DOI: 10.1021/la800551u), **24**, 6759.
32. R. C. Ritter, *Physiol. Behav.*, 2004, **81**, 249.

CHAPTER 5

Nanotechnology Applications for Food Ingredients, Additives and Supplements

QASIM CHAUDHRY[a] AND KATHY GROVES[b]

[a] The Food and Environment Research Agency, Sand Hutton, York YO41 1LZ, UK; [b] Leatherhead Food International, Leatherhead Surrey, UK

5.1 Introduction

The current applications of nanotechnology in the food sector are few, as the science is relatively newly emergent, but they have been on a steady rise in recent years, and are predicted to grow rapidly in the coming years. This is because the new technologies have a great potential to address many of the food industry's current needs.

The main benefits of nanotechnology applications derive from the improved or novel functionalities of nano-sized materials and substances that have a much larger surface to mass ratio compared to the bulk equivalents. The very small size of nanomaterials enables dispersion of water-insoluble additives (such as colours, flavours and preservatives) without the need for additional fat or surfactants. Nano-sized substances are also claimed to have greater uptake, absorption and bioavailability in the body compared to bulk equivalents, opening a way for the use of a variety of nano-sized ingredients, supplements and nutraceuticals in (health)food applications. The processing of food ingredients at a nanoscale is thus enabling the development of improved or new tastes, flavours, textures and nutritional value. Nano-sized additives are also

RSC Nanoscience & Nanotechnology No. 14
Nanotechnologies in Food
Edited by Qasim Chaudhry, Laurence Castle and Richard Watkins

Published by the Royal Society of Chemistry, www.rsc.org

used to improve safety, traceability and shelf-life of certain food products. In spite of such prospects, the use of nanotechnologies in consumer products has raised a number of new health and environmental safety, ethical, policy and regulatory issues. Predictably, such applications in food are attracting a greater public concern than those in other consumer sectors.

This chapter is aimed at presenting and discussing state-of-the-art in regard to applications of nanotechnologies for food ingredients, additives and supplements. The information has been collated from a variety of sources that include published literature, company websites, patent databases, national and international inventories, market analysis reports, key scientific reviews and reports, material presented at relevant conferences, workshops and symposia, and through contacts with leading experts in the area of food nanotechnology.

5.2 Current Status of Nanotechnologies and Future Trends

A number of recent reports and reviews have identified the current and short-term projected applications of nanotechnologies for food and beverages.[1–6] Like many other sectors, nanotechnologies are promising to revolutionise the food sector – from production to processing, storage and development of innovative materials, products and applications. Although food applications of nanotechnologies are relatively newly emergent, it is evident from the available market reports, patent applications and the increasing number of products that they have started to make an impact on the food sector. The number and range of nanotechnology derived food products is further expected to increase in the coming years. According to a market analysis report by Helmut Kaiser Consultancy,[7] the nanofood sector is currently led by the USA, followed by Japan and China; but the report expects Asian countries (led by China) to be the biggest market for nanofood by 2010. Currently, most of the developments in food area are outside Europe, although some nanotechnology-derived supplements and food packaging materials are available in the EU. The global nature of business in the food and related sectors, however, means that an increasing number of products and applications is likely to be available in the future to consumers worldwide.

Estimates of the current global market size and number of companies involved in the nanofood sector are varied because of the scarcity of exact information due to certain commercial and other sensitivities. This makes it difficult to gather information on the true commercial activity in this area. A lot of the available information in 'grey' literature seems to be aimed at projecting the 'magic' of nanotechnologies, rather than the factual products and applications that are either available or are near-market. In view of this, the information presented in this chapter has been sifted in an attempt to separate facts from fiction, and the products and applications that have been mentioned are those which are (or are likely to be) available, whilst still keeping track of those that can be regarded as 'anticipated' at this stage. The overall size of market for

nanofood has been estimated at around US$7 billion in 2006, predicted to grow to over US$20 billion by 2015.[8] Another report by the consulting firm Cientifica[8] has estimated the then current (2006) food applications of nanotechnologies at around $410m (food processing US$100m, food ingredients US$100m and food packaging $210m). According to the report, the existing applications are mainly for improved food packaging, with some applications for delivery systems for nutraceuticals. The report estimated that by 2012 the overall market value would reach $5.8 billion (food processing $1303m, food ingredients $1475m, food safety $97m and food packaging $2.93 billion). Considering such rapid developments in this field, and the global set-up of major food companies, it is not unreasonable to anticipate that nanofood products will be increasingly available on the markets worldwide in the coming years.

It has been suggested[9] that the number of companies currently applying nanotechnologies to food could be as high as 400. Many international food and beverage companies are reported to have an active interest in nanotechnology, although information relating to the actual scale of commercial activity in this area is scarce.

5.3 Current and Projected Applications

An overview of the main developments in the nanofood[9] area indicates that they have so far been mainly aimed at packaging and food supplements. Applications for food products or ingredients are few to date but intentions are to look at processing food components to develop new textures, using nano-sized (or nano-encapsulated) food ingredients and additives to develop new tastes, textures and sensations, and to control the release of flavours and/or increase bioavailability of nutrients and supplements.

A closer look at the applications suggests that many of these have emerged from similar technologies developed in related sectors, in particular pharmaceuticals and cosmetics. This is because there is an overlap between food, medicine and cosmetics sectors in the areas of health foods and nutraceuticals, cosmeceuticals and nutricosmetics. The cross-cutting nature of nanotechnologies means that materials and applications developed in one of the sectors is also finding use in the related sectors. In this regard, some food and cosmetic companies are known to be collaborating to develop cosmetic-nutritional supplements.[9]

The current and short-term projected applications of nanotechnologies in the (health)food sector include nano-sized or nano-encapsulated ingredients and additives for food, beverage and health food applications. A current niche for such applications is where some food products are marketed as a means to enhance nutrition for different lifestyles, or as an aid to beauty, health and wellbeing. These areas are the first focus of nanotechnology applications, which have gradually started to appear in the mainstream food sector. Thus the vast majority of the currently available nanotechnology products are in the areas of

supplements, health foods and nutraceuticals, with only a few products in the food and beverage areas. A summary of the broad categories of nano-technology applications in the (health)food sectors is provided in Table 5.1.

The main tenet behind the development of nano-sized food ingredients and additives appears to be the enhanced uptake and bioavailability of nano-sized substances in the body, although other benefits such as improvement in taste, consistency, stability and texture, *etc*. have also been claimed.[3] Recent reviews and reports[2 7,9] have identified the following main categories of known and projected applications of nanotechnology in the (health)food area:

- where food ingredients have been processed or formulated to form nanostructures for enhanced activity, protection from degradation or novel functionality
- where nano-sized, nano-encapsulated or engineered nanoparticle (ENP) additives have been used in food
- indirect applications, such as nanofiltration for the removal of undesirable components such as allergens in food, and sensors for food safety.

An understanding of the current R&D activities in the area of nanofood can also provide an insight into the possible future developments. It has been estimated that over 200 companies worldwide are conducting R&D into the use of nanotechnology in engineering, processing, packaging or delivering food and nutritional supplements.[9,10] Whilst only a handful of (health)food products containing nano-additives are currently available, it has been estimated that over 150 applications of nanotechnology in food may be at different stages of development.[9] A search of patent databases found more than 400 patent entries with regard to applications of nanotechnology in food or food contact materials. The main R&D themes in this area are aimed at:

- reducing the amount of salt, fat, colour or other additives to promote healthy option foods
- improving the properties of food, e.g. by altering colour, flavour, texture, consistency, and developing new tastes and sensations in the mouth, and producing better sensory and stability properties
- controlling the release of flavours and nutrients, and enhancing the absorption of nutrients and nutraceuticals in the body
- developing new sensors for rapid detection of bacteria or viruses, or for smart packaging to sense when a food product has past the use-by time
- introducing novel surface coatings both to packaging and to processing equipment to give enhanced properties.

The current R&D efforts are, however, mainly focused at the development of high-value products, such as nutraceuticals, interactive and functional foods. These include enabling the consumers to modify their food depending on their choice, needs or tastes. A projected example of the latter is a colourless and tasteless beverage that would contain nano-encapsulated ingredients or

additives that could be activated by a consumer at a particular microwave frequency. This would lead to activation of selected nanocapsules, whilst the others remain intact, releasing only the preferred flavour, colour or nutrients.[9]

5.4 Nanomaterials for (Health)food Applications

The nanomaterials currently used in (health)food applications include both inorganic (metal, metal oxides) and organic (often natural) materials. In addition to the purposely manufactured nanomaterials, there is a possibility that certain microscale materials used in food may contain a nanoscale fraction due to the natural size range variation.[4] Based on the available information, the nanomaterials likely to be used in (health)food applications fall into three categories:

- metal and metal oxides (including alkaline earth metals) and non-metals
- surface functionalised nanomaterials
- organic nanomaterials.

Examples of these are given in the following sections.

5.4.1 Metal/Metal Oxides

A number of metal and metal oxide ENPs are known to be used in (health)food products and food packaging applications. These include ENPs of transition metals such as silver and iron; alkaline earth metals such as calcium and magnesium; and non-metals such as selenium and silicates. Others ENPs that can potentially be used in food applications include titanium dioxide. Food packaging is the major area of application of metal(oxide) ENPs. Examples include plastic polymers with nanoclay as gas barrier, nano-silver and nano-zinc oxide for antimicrobial action, nano-titanium dioxide for UV protection, nano-titanium nitride for mechanical strength and as a processing aid, nano-silica for surface coating, etc. (Chapter 6).

Among the currently used ENPs, nano-silver is finding an increasing use in consumer products, such as medical applications, cosmetics and personal care products, and food packaging materials. The use of nano-silver has also been suggested for a variety of health(food) supplements for natural antimicrobial action. Nano-silver formulations are now available from a number of sources, and its use in (health)food applications is likely to increase in the future. Although no clear-cut food product containing nano-silver is currently available, its use as an additive to prepare antibacterial wheat flour, containing 100–1000 ppm of silver nanoparticles of around 10 nm has been the subject of a recent patent application.[11]

Nano-silica is known to be used in food contact surfaces and food packaging applications, and some reports suggest its use in clearing of beers and wines, and as a free flowing agent in some powdered soups. The conventional bulk

Table 5.1 Summary of nanotechnology applications for (health)food products.

Application area	*Nanotechnology/function*	*Likely benefits*	*Potential risk*	*Availability*
Nano-structured* food ingredients * also termed as nano-textured.	Processed nanostructures in food novel or improved tastes, flavours, textures.	Use of less fat, better tasting food products, more stable emulsions.	The nanostructures are likely to break down in the gastrointestinal (GI) tract, and hence there may not be a risk to consumer health.	Currently, there is no clear example of a proclaimed nano-structured food product that is commercially available, although some products are known to be at different stages of R&D – some of them may be nearing market.
Nano-delivery systems for nutrients and supplements.	Nano-encapsulated bioactive substances in the form of nanomicelles, liposomes or protein-based carrier systems – mainly additives and supplements for food and beverage products.	Preservation of ingredients and additives during processing and storage, masking unpleasant tastes and flavours, controlling the release of additives, and enhanced uptake of the encapsulated nutrients and supplements.	If the nanocarriers break down and release their contents in the GI tract, any risk posed by the encapsulated substance should not be any different from the conventional bulk equivalents. If, however, a nanocarrier is capable of delivering the substance to the bloodstream, its ADME (absorption, distribution, metabolism, excretion) characteristics and potential effects may be different from the conventional form.	A number of nanocarrier based delivery systems are available with a range of encapsulated materials. Examples include food additives (e.g. benzoic acid, citric acid, ascorbic acid), and food supplements (e.g. vitamins A and E, isoflavones, β-carotene, lutein, omega-3 fatty acids, coenzyme-Q10).

Organic nano-sized additives for food and health food applications.	Organic additives (many of them naturally occurring substances) manufactured in the nano-size range. Due to increased surface area, a smaller amount would be needed for a function, or taste attributes.	The main advantage is claimed to be the better dispersability of water-insoluble additives in food-stuffs without the use of additional fat or surfac-tants, and enhanced tastes and flavours due to enlarged surface areas of the nano-sized additives compared to conventional bulk forms. Products in this category are also claimed for enhanced absorption and bioavail-ability of nanosubstances in the body compared to conventional equivalents.	A greater bioavailability of certain substances may pose a risk to consumer health, but this will depend on the extent and frequency of the exposure(s), and whether the nanomaterials are assimilated in and outside the GI tract in a different manner compared to the conventional forms.	This type of application is expected to exploit a much larger segment of the (health)food sector, encompassing colours, preservatives, flavourings and supplements. A range of products containing nano-sized additives is already available in the supplements, nutraceu-ticals and (health)food sectors. Examples include vitamins, colorants, flavouring agents, antioxidants, *etc*.
Inorganic nano-sized additives for food, and health food applications.	Inorganic additives manu-factured in the nano-size range. Due to a large surface area, a smaller amount would be needed for a function or a taste attribute. Other projected benefits include enhanced uptake of supplements, and health benefits due to the antimicrobial activity of some nanomaterials.	Enhanced tastes and flavours due to enlarged surface areas of the nano-sized additives over conventional forms. Products in this category are also claimed for enhanced absorption and bioavailability in the body compared to conven-tional equivalents.	Potential risk of exposure to insoluble, indigestible and/ or bio-persistent nanomaterials	A range of inorganic additives is available in the supplements, nutraceu-ticals and (health)food sectors. These include metals, metal oxides, alkaline earth metals, non-metals, as well as surface functionalised materials. Examples include silver, iron, silica, titanium dioxide, selenium, calcium, magnesium *etc*.

form of silica is a permitted food additive (SiO_2, E551), but concerns have been raised over the safety of nano-silica since it is likely to remain undigested in the GI tract and may be translocated to other parts of the body.

Titanium dioxide, in conventional bulk form, is an already approved additive for food use (TiO_2, E171), but there is the possibility that the conventional form may also contain a nano-sized fraction. Nano-titanium dioxide is currently used in a number of non-food consumer products (e.g. paints, coatings), and its use may extend to foodstuffs in the future. A projected example of this is for coating of confectionery products[12] that include the permitted additives silicon dioxide (SiO_2, E551), magnesium oxide (MgO, E530) and titanium dioxide (TiO_2, E171), which are preferably insoluble. The coating is intended for application in a continuous process as a thin amorphous film of 50 nm or less, to provide moisture or oxygen barriers and thereby improve shelf life and/or the flavour impact of confectionery products.

5.4.2 Surface Functionalised Nanomaterials

Surface functionalised nanoparticles are the second generation nanoparticles that add certain functionality to the matrix, such as antimicrobial activity, or a preservative action through absorption of oxygen. For food packaging materials, functionalised ENPs are used to bind with the polymer matrix to offer mechanical strength or a barrier against movement of gases, volatile components (such as flavours) or moisture. Compared to inert materials, the use of this category of ENPs in food applications is likely to grow in the future. They are also more likely to react with different food components, or become bound to food matrices, and hence not available for translocation out of the GI tract. Examples include organically modified nanoclays that are currently used in food packaging to enhance gas-barrier properties.

5.4.3 Organic Nano-additives and Processed Nanostructures in Food

A number of organic nano-sized materials are used (or have been developed for use) in food products. These include supplements (e.g. vitamins, antioxidants), colours, flavours and preservatives. The main principle behind the development of nano-sized organic substances is the greater uptake, absorption and bioavailability in the body, compared to conventional bulk equivalents. However, a greater uptake and bioavailability of certain compounds, such as preservatives, could pose a greater risk to consumer health and this needs to be further investigated through research.

One example of an organic food additive is the synthetic form of lycopene, a carotenoid found in tomato that has very good colorant and antioxidant properties. A synthetic form of lycopene is reported to have a particle size in the range of 100 nm.[13] The insolubility of carotenoids in water, moderate solubility in fats and oils, and susceptibility to oxidation, impede the direct use of

relatively coarse particles, which also limits their colouring ability. The nanoparticulate nature of the synthetic lycopene is intended to offer a wide diversity of colouring properties, with improved bioavailability. The possible foodstuff applications include soft drinks, baking mixtures and blancmanges but this is predicted to expand in scope significantly.

Also developed for use in food products are nano-sized carrier systems for nutrients and supplements. These are based on nano-encapsulation of the substances both in liposomes and micelles, as well as protein-based carriers. Such nanocarrier systems are used for taste masking of ingredients and additives such as fish oils, and protection from degradation during processing. They are also claimed for enhanced bioavailability of nutrients, supplements, antimicrobial activity and other health benefits. There is a wide range of materials available in this category, for example, food additives (e.g. benzoic acid, citric acid, ascorbic acid), and supplements (e.g. vitamins A and E, isoflavones, β-carotene, lutein, omega-3 fatty acids, coenzyme-Q10). Examples include Novasol® (Aquanova®, Germany), and 'nano-sized self-assembled liquid structures (NSSL)' (NutraLease Ltd., Israel). An example of NSSL-based product is 'Canola Active Oil' (Shemen Industries, Israel), which is fortified with supplements, such as phytochemicals. A similar technology is based on NanoCluster™ delivery system for food products (RBC Life Sciences® Inc., USA). A number of products has been developed based on NanoCluster™ system, including 'Slim Shake Chocolate' that is reported to contain cocoa nanoclusters. The product is understood to incorporate nano-sized silica particles that are coated with cocoa to enhance the chocolate flavour through the increase in surface area that hits the taste buds. BioDelivery Sciences International has developed Bioral™ nanocochleate nutrient delivery system, for micronutrients and antioxidants. This phosphatidylserine based carrier system ($\sim$50 nm) is derived from soybean, generally regarded as safe (GRAS). The BioDelivery Sciences International's Bioral™ nanocochleate is a nutrient delivery system for protecting micronutrients and antioxidants from degradation during manufacture and storage. The system is claimed to have enabled the addition of omega-3 fatty acids for use in goods that are then baked or cooked such as cakes, muffins, pasta noodles, soups and cookies. The company has also claimed to have added the Bioral™ omega-3 formulation to soy milk, milk, liquid yoghurt, orange juice, smoothies, sports drinks, soft drinks, coffee, frappuccinos and other beverages without altering taste or odour.

Self-assembled nanotubes from hydrolysed milk protein α-lactalbumin with a good stability have recently been reported.[14] σ-Lactalbumin is already used as a food ingredient, mainly in infant formula products. These food-protein derived nanotubes may provide a new carrier for nano-encapsulation of nutrients, supplements and pharmaceuticals.

The concept of nanodelivery systems has essentially originated from research into targeted delivery of drugs and therapeutics. The use of similar concepts in foodstuffs is interesting because whilst nanocarrier systems can offer increased absorption, uptake and bioavailability of a compound, it also has the potential in theory to alter tissue distribution of the substances in the body. For example,

the technology can turn water-soluble substances (such as vitamin-C) into fat-dispersible forms through nanocarrier technology, and vice versa, can turn fat-dispersible substances (e.g. vitamin A) into water-dispersible forms. If the nanocarrier is completely broken down and releases the contents in the GI tract, any risks posed by the encapsulated compound should not be any different from the conventional bulk equivalent. However, if a nanocarrier system is capable of delivering the encapsulated substance to the bloodstream, its absorption, tissue distribution and bioavailability (and hence the health risk) might be very different from the conventional forms.

Current research is also aiming at the development of nano-structured (or nano-textured) food ingredients and additives to improve taste, colour, flavour, texture and consistency. For example, nano-structured mayonnaises, spreads and ice-creams may have a creamy texture with much less (or no additional) fat, and hence will offer a healthier option to the consumer. The processes commonly used for producing nano-structured food products include nano-emulsions, surfactant micelles, emulsion bilayers, double or multiple emulsions and reverse micelles.[15] An important point to mention is that all foods contain nanoparticles and the processing of ingredients or foods themselves can change the structure so that the properties such as taste, texture and stability are improved. The changes need not necessarily involve a reduction in size to the nanoscale as it has been found that reduction to the microscale can also produce large and improved changes to the functional properties of an ingredient. Changes to properties of all ingredients could be found by reducing the size to the micrometer or nanometer level and this could lead to a reduction in the need for current levels of certain ingredients or improvements to ingredient functionality generally. Two areas that have been found promising are the change in size or structure of salt to enable the industry to lower the salt levels in foods while maintaining taste, functionality and safety, all of which are actions of salt in foods; and the restructuring of water in emulsions to improve the properties of low-fat products such as dressings.

Figure 5.1 shows the surface of a potato chip with table salt added as usual by the consumer. Studies from a project on sensory properties of different salt sizes at Leatherhead Food International showed that reducing the size to 10–40 μm increased the initial salt time and intensity. The micrograph illustrates that table salt with its large size does not cover much of the surface of the chip (or other product). The use of a smaller sized salt would give more salt particles on the product and allow the consumer to taste the salt more when added at a lower level.

In reduced-fat emulsion products such as salad dressings, the extra water needs to be stabilised with added ingredients such as modified starch to maintain stability and viscosity. Restructuring the water to enclose it within the fat droplets by manufacturing a water in oil in water (WOW) emulsion allows the lower fat product to maintain the higher stability and creaminess associated with a higher fat product. An example of the structure of the WOW emulsion is shown in Figure 5.2. In this confocal microscope image, the oil appears white and the water black. This work was produced in a LINK project between

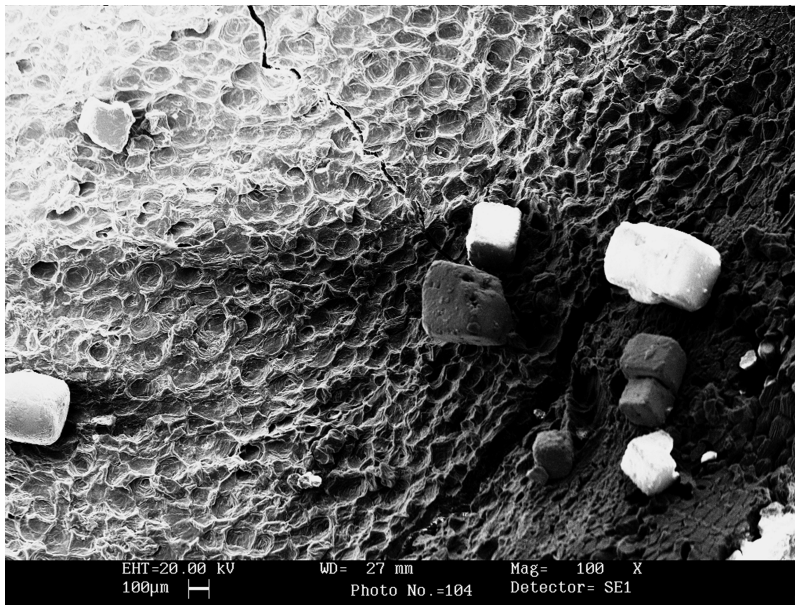

Figure 5.1 Scanning electron micrograph of table salt on the surface of a potato chip.

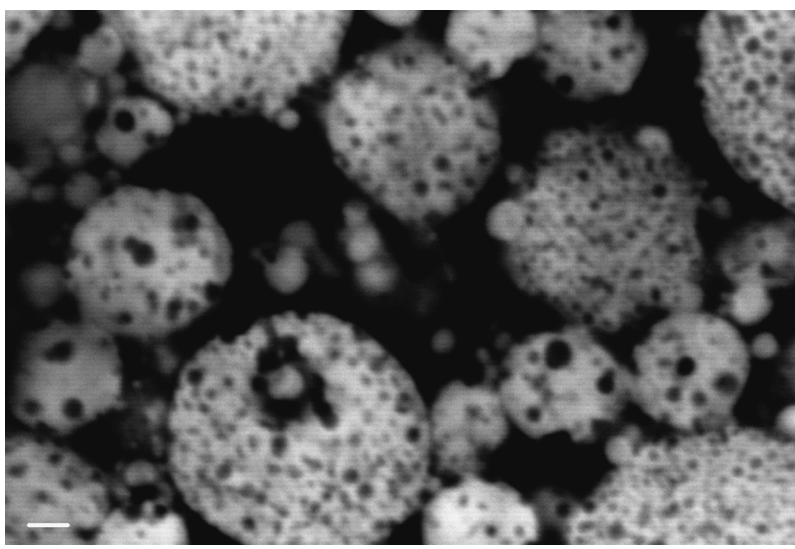

Figure 5.2 Confocal scanning laser micrograph of a WOW emulsion: water = black, oil = white, bar = 2 µm.

Leatherhead Food International, Institute of Food Research, DEFRA and industry.[16]

Interesting developments might be seen if emulsions were produced with all nano-sized oil droplets. It is possible that they could be stable without the need for current emulsifiers.

It is also worth mentioning that many other nanomaterials are used in non-food applications, but they are unlikely to find applications in food or food packaging. Examples of these include carbon-based materials (such as fullerenes and carbon nanotubes). Recent studies have linked carbon nanotubes with potential harmful effects in biological systems.[17] However, they are highly unlikely to be used in food applications, because the functionalities they offer relate to enhanced tensile strength and electrical conductivity, which are of little benefit for use in food. Application of such materials in the packaging area is, nevertheless, a possibility.

5.5 Nano-sized Food Ingredients and Additives in Relation to Digestion of Food

The main likely route of entry of micro- or nano-sized particles to the gut is through consumption of food and drinks. The main health concerns will, therefore, be dependent on the physicochemical nature of the nanomaterials in food products, and the extent and duration of the consumer exposure through consumption of the nanofoods. It is well known that a healthy digestive system only allows absorption of nutrients from the gut after digestion of foodstuffs. The gut wall is designed to ensure the passage of dietary nutrients, and prevent the passage of larger or foreign materials. At the cellular level, the transport of conventional forms of nutrients (and metabolites) is also well regulated. Because of the very small size, and depending on the surface charge and coatings, *etc*., nanomaterials may 'override' these mechanisms and end up in other non-intended tissues and organs, potentially posing a health risk to the consumer (see Chapter 8 for further discussion).

It is worth highlighting that a naturally occurring or synthetic nanomaterial that is not bio-persistent, i.e. it is either solubilised, assimilated in the gut or at cellular level, or excreted from the body, is not likely to pose any different health risk than the conventional bulk equivalent. On the other hand, the use of insoluble/indigestible ENPs in food applications may raise certain health concerns. The presence in food of such ENPs, such as silver, titanium dioxide or silica to name a few, poses the likelihood of translocation of potentially large reactive surfaces to different parts of the body that could have health implications. It is, therefore, important to understand how insoluble particulate materials are handled by the digestive system. It is known that many food substances exist naturally, or are metabolised in the body at a nanoscale.

Many globular food proteins are reported to be between tens to hundreds of nanometers in size, and most polysaccharides and lipids are linear polymers less than 2 nm in thickness.[18] The three main constituents of food (proteins,

carbohydrates and lipids) are each digested in a different manner. However, a common factor between the three is that digestion of their constituents occurs at the nanoscale. Based on this, it could be argued that the processing of foods at the nanoscale would simply improve the speed or efficiency of their digestion, uptake, bioavailability and metabolism in the body. Indeed, within the nutrition market there are already supplements that claim to contain di- and tripeptides, and are thus more readily digestible.[19] In contrast, it could be argued that since the processing of substances to this scale often alters their properties, then nanoscale processing of foods may alter how the food ingredients 'behave' upon breakdown within the gut, and as a consequence how they are treated in the gastrointestinal tract.

The intestinal wall is folded into villi to maximise the surface area for digestion. The villi surface is composed of two main cell types: enterocytes (the majority) and goblet cells. Translocation of particles through the intestine depends on four main factors:

1. diffusion and accessibility through mucus lining the gut wall
2. initial contact with enterocytes or M-cells
3. cellular transport
4. post-translocation events.[20]

5.5.1 Translocation of Particulates Through Intestinal Mucus

A mucus layer lines the epithelial cells of the gut wall. It is secreted by goblet cells, one of the two principal cell types found in the intestinal wall. Mucus is principally composed of proteins (called mucins) within an electrolyte suspension[21] and helps to trap pathogens and remove foreign materials before they come into contact with the gut epithelium. Passage of particulates through the intestinal mucus is dependant on multiple factors, two of these being particle size and charge.

The mucus lining the gastrointestinal epithelia forms a mesh-like barrier through which it was originally thought that passage of molecules over ~ 55 nm in diameter was prevented. This ensures that only small molecules could have access the villi of the gut epithelium for digestion. It has also been demonstrated that smaller particles are able to diffuse through the mucus layer faster than larger particles (see Chapter 8). Passage of particles through intestinal mucus is dependant on surface charge. Szentkuti[22] showed that particles of various sizes and charges diffused through intestinal mucus at differing rates. Cationic nanoparticles were found to become entrapped within the negatively charged mucus, whereas carboxylated (anionic) microparticles were able to diffuse successfully through to the epithelial surface. It has also been discovered recently that pores within the mucus layer are much larger than originally anticipated. Researchers at the Johns Hopkins University, Maryland, have published evidence to suggest that particles as big as 200 nm can pass through mucus pores when coated with polyethylene glycol, a substance used commonly

to coat drug particles and to prevent uptake by phagocytic immune cells.[23] This was due to the neutral surface charge of the particles coated with polyethylene glycol.

5.5.2 Contact with Enterocytes and M-cells

Absorption of food occurs mainly through enterocytes situated on the villi of the gut wall epithelium. Enterocytes serve two main functions:

1. to control passage of macromolecules and pathogens
2. to allow absorption of dietary constituents.

Contained within both intestinal mucus and gut wall epithelium are aggregates of lymphoid nodules, commonly referred to as Peyer's patches, and within the epithelium of Peyer's patches are M-cells. These take in samples of foreign material and deliver them to underlying lymphocytes to elicit immune responses and thus control disease.[24] It is here that foreign objects that have passed through intestinal mucus are usually accumulated.

Under normal circumstances, passage of particles through enterocytes takes place after foodstuffs have been digested into their constituents, and this process may be passive, facilitated or active depending on the characteristics of the breakdown product.

5.5.3 Cellular Translocation

Once food constituents have been broken down by enzymes, diffused through the gut mucosa, and absorbed through the enterocytes of the epithelia, they are translocated across the cells and pass into the hepatic circulation. There are several mechanisms by which particles may pass through the epithelia. These are:

1. transcytosis by enterocytes (as with normal digestion)
2. transcytosis by M-cells (although this is more likely to lead to accumulation within M-cells, and a consequent immune reaction)
3. passive diffusion across the epithelia or paracellular transport.

The time between the initial contact of particulates with the epithelial wall, to their absorption and translocation across cells is relatively slow. Szentkuti[23] reported that accumulation of particles within the cell layer under the intestinal epithelium was still relatively low after several days of oral gavage of particles within rats.

Translocation through intestinal epithelia occurs by transcytosis through enterocytes. This is the basis of normal absorption (e.g. selective uptake of peptides or amino acids through transporters within the brush border), and it is well documented that gastrointestinal uptake of exogenous nanoparticles is

greater than microparticles. Desai *et al.*[25] showed that translocation of nanoparticles, 100 nm in diameter, is 15–250 times greater than that shown by micromolecules, which are more likely to become lodged within Peyer's patches.[22] The gastrointestinal uptake of nanoparticles has been shown to be 2–200 times greater on Peyer's patches, despite the fact that these only represent ~1% of the total intestinal surface.[22]

Translocation of manufactured nanoparticles through the epithelium is likely to be dependent on the physiochemical properties of the nanoparticle, e.g. zeta potential, hydrophobicity, size, presence/absence of a ligand and the physiology of the intestinal tract, e.g. healthy or diseased state (where translocation may be increased or decreased, depending on the illness).[22]

Under normal physiological conditions, paracellular transport of nanoparticles would be extremely limited, as pore size at tight junctions is between 0.3 nm and 1.0 nm.[22] However, research into improving paracellular transport through gut epithelia is being carried out alongside medical research into targeted drug delivery. For example, research is being carried out into using positively charged poly(acrylic) acids to aid nanoparticle passage via interaction with the negatively charged surface of the epithelium, or complexing Ca^{2+} involved in the structure of tight junctions.[22]

Despite the few published studies, there are a number of knowledge gaps in regard to the behaviour, interactions, fate and effects of ENPs inside and outside the GI tract. Any health risk of a nanomaterials in food will be dependent on the level and frequency of the exposure and on how the ENPs are dealt with, both inside and outside the GI tract. Due to the current unavailability of appropriate analytical methodologies, it is not known whether any ENPs added to food will remain in a free form in the GI tract, and thus available for translocation. From the enormous free surface energies, it can be anticipated that most ENPs will not remain in a free form in foodstuffs or in the GI tract because of agglomeration, binding with other food components, *etc*. They are also likely to undergo degradation or certain transformations in the GI tract, e.g. through reaction with stomach acid and digestive enzymes, absorption/binding of different moieties on surface, *etc*. These transformations are likely to further affect the translocation and internal exposure to free ENPs.

5.6 Conclusions

Nanotechnology applications for the food and related sectors are currently at an elementary stage, but are set for a predictable growth in the future. As with any new technology, most of the current and short-term predicted applications of nanotechnologies are for high-value products. An increasing number of nanotechnology based (health)food products is available to consumers worldwide, and the range and number of products is predicted to increase in the coming years. Nanotechnology applications for food ingredients and additives do offer a range of benefits to the consumer, in terms of innovative, tasteful and healthy food products. There are certain concerns over the use of insoluble,

indigestible and bio-persistent nanomaterials in food as they might pose a risk to consumers' health. Whilst such applications will need thorough safety assessments and further research, it is likely that the use of soluble, digestible and non-persistent materials, such as food nanostructures and nano-emulsions, will spearhead the way for the new technological innovations in the (health)-food sectors.

References

1. H. Bouwmeester, S. Dekkers, M. Noordam, W. Hagens, A. Bulder, C. de Heer, S. ten Voorde, S.Wijnhoven and A. Sips, *Health Impact of Nanotechnologies in Food Production*, Report 2007.014, RIKILT – Institute of Food Safety, Wageningen UR and National Institute of Public Health & the Environment; Center for Substances and Integrated Risk Assessment, 2007.
2. Q. Chaudhry, M. Scotter, J. Blackburn, B. Ross, A. Boxall, L. Castle, R. Aitken and R. Watkins, *Food Addit. Contam.*, 2008, **25**(3), 241–258.
3. EFSA, Scientific Opinion on 'The Potential Risks Arising from Nanoscience and Nanotechnologies on Food and Feed Safety', adopted on 10 February 2009.
4. Food Safety Authority of Ireland, *The Relevance for Food Safety of Applications of Nanotechnology in the Food and Feed Industries*, Food Safety Authority of Ireland, Abbey Court, Lower Abbey Street, Dublin 1, 2008.
5. K. Groves, *New Food Mag.*, 2008, **4**, 49–52.
6. V. J. Morris, *New Food Mag.*, 2008, **4**, 53–55.
7. Helmut Kaiser Consultancy, *Study: Nanotechnology in Food and Food Processing Industry Worldwide*, 2003–2006–2010–2015, www.hkc22.com/Nanofood.html, 2004 (accessed February 2007).
8. Cientifica Report, *Nanotechnologies in the Food Industry*, published August 2006. Available at: www.cientifica.com/www/details.php?id=47, 2006 (accessed 24 October 2006).
9. The recently coined term 'nanofood' refers to the use of nanotechnology techniques, materials or tools during production, processing, or packaging of food.
10. Institute of Food Science and Technology (IFST) Trust Fund, 2006, Nanotechnology information statement, www.ifst.org/uploadedfiles/cms/store/attachments/nanotechnology.pdf.
11. K. H. Park, Preparation method antibacterial wheat flour by using silver nanoparticles, Korean Intellectual Property Office (KIPO), Publication number/date 1020050101529A/ 24.10.2005.
12. Mars Inc., Edible products having inorganic coatings, US Patent US5741505, www.freepatentsonline.com/US5741505.html.
13. BASF, Production of carotenoid preparations in the form of coldwater-dispersible powders, and the use of the novel carotenoid pigments, US Patent US5968251, www.freepatentsonline.com/US5968251.html.

14. J. F. Graveland-Bikker and C. G. de Kruif, *Trends Food Sci. Technol.*, 2006, **17**(5), 196–203.
15. J. Weiss, P. Takhistov and D. J. McClements, *J. Food Sci.*, 2006, **71**(9), R107–R116.
16. S. M. Clegg, A. I. Knight, C. J. M. Beeren and P. J. Wilde, Fat reduction whilst maintaining the sensory characteristics of fat using multiple emulsions, 5th International Symposium on Food Rheology and Structure (ISFRS, 2009), pp. 238–241.
17. C. A. Poland, R. Duffin, I. Kinloch, A. Maynard, W. A. H. Wallace, A. Seaton, V. Stone, S. Brown, W. MacNee and K. Donaldson, *Nature Nanotechnology*, 2008, **3**, 423–428.
18. www.ifr.ac.uk/publications/scienceinnovation/0502_nanotechnology/.
19. L. Crisalle, Types of Protein and Differences in Quality, January 2007, www.exerciseandnutritionworks.com/t-protowhey1.aspx.
20. P. H. M. Hoet, I. Brüske-Hohlfeld and O. V. Salata, *J. Nanobiotechnology*, 2004, **2**, 12.
21. A. des Rieux, V. Fievez, M. Garinot, Y.-J. Schneider and V. Préat, *J. Controlled Release*, 2006, **116**, 1–27.
22. L. Szentkuti, *J. Controlled Release*, 1997, **46**, 233–242.
23. K. Samuel, D. Lai, E. O'Hanlon, S. Harrold, S. T. Man, Y. -Y. Wang, R. Cone and J. Hanes, *Proc. Natl. Acad. Sci.*, 2007, **104**, 1482–1487.
24. R. M. Berne and M. N. Levy, *Principles of Physiology,* Mosby Inc, Missouri, 3rd edn., 2000.
25. M. P. Desai, V. Labhasetwar, G. L. Amidon and R. J. Levy, *Pharm. Res.*, 1996, **13**, 1838–1845.

CHAPTER 6

Nanotechnologies in Food Packaging

MARIA SMOLANDER[a] AND QASIM CHAUDHRY[b]

[a] VTT Technical Research Centre of Finland, Tietotie 2, Espoo, P.O. Box 1000, FI-02044 VTT, Finland; [b] The Food and Environment Research Agency, Sand Hutton, York YO41 1LZ, UK

6.1 Introduction

The main function of food packaging is to maintain the quality and safety of food and beverage products during storage and transportation, and to extend the shelf-life by controlling the permeation of moisture, gases and other volatile components such as flavours and taints. One of the current major global challenges is to address our over-stretched food resources for the growing population worldwide and the need to reduce the amount of food that is wasted. Appropriate quality packaging, along with food quality and safety indicators, can play a major role in this regard. The food and beverage industry has therefore always been seeking new technologies, including efficient gas-barrier materials, to improve quality, shelf-life, safety and traceability of their products. Packaging of food products has evolved over the years from wooden crates, cardboard boxes and cellophane wrappings to new stronger but light-weight, recyclable and functional packaging materials. Food packaging is now also associated with other features that relate to the active improvement and/or maintenance of the product quality, and to the measurement, storage and distribution of information about the product. These features have been termed 'active' and 'intelligent' (or 'smart') packaging. Packaging concepts and

RSC Nanoscience & Nanotechnology No. 14
Nanotechnologies in Food
Edited by Qasim Chaudhry, Laurence Castle and Richard Watkins

Published by the Royal Society of Chemistry, www.rsc.org

materials with functionalities such as oxygen scavenging capacity, antimicrobial activity, light-barrier properties, indication of food quality and indication of product authenticity, etc. offer new possibilities for the maintenance of quality and safety of food products.

As in other sectors, the advent of nanotechnology has opened up a way for innovations in the food packaging field in recent years. Innovative packaging is predicted to be one of the fastest growing areas of the application of nanotechnologies in the food sector. Indeed, nanotechnology-derived food packaging makes up the largest share of the current and short-term predicted nanofood sector. Cientifica (2006)[1] has estimated that food packaging forms approximately half of the total value of the current applications of nanotechnologies by the food sector. The report also estimated that by 2012 the overall value of the nanofood market would reach $US 5.8 billion, with food packaging being one-half at $US 2.9 billion. Other forecasts, such as by Nanoposts (2008), have estimated that nanotechnology-derived packaging (including food packaging) will make up to 19% of the share of nanotechnology products and applications in the global consumer goods industry by 2015. Whether or not these upbeat forecasts turn out to be accurate, they nevertheless reflect the considerable opportunities that exist for nanotechnologies to make major technical impacts on food packaging materials.

This chapter will describe the prospects of nanotechnology for the improvement of properties such as stability and barrier properties of both conventional packaging materials and biodegradable polymers. These properties include rigidity, stiffness or flexibility, durability, temperature and moisture stability, and barrier properties against light, oxygen and other gasses. Additionally, active packaging materials that incorporate engineered nanoparticles (ENPs) or nanolayers with antimicrobial or oxygen scavenging properties will be described. Further on, intelligent (or smart) food packaging, incorporating sensors and indicators to monitor the condition of the food or to indicate the authenticity of the packaged product will be discussed as potential application area for nanotechnology. Finally, safety evaluation and the potential migration of nanoparticles from food contact materials in packaging applications will be discussed, together with the future direction of developments in this area.

6.2 Improvement of Mechanical Properties through Nanocomposites

Nanoparticle-reinforced materials (also termed as 'nanocomposites') are polymers reinforced with nanoparticles to provide a composite material with enhanced properties. Unlike some conventional fillers, such as glass fibres and talc, only a low level of nanoparticles is usually sufficient to enhance the properties of the composite materials.[2] The composites are reinforced with small quantities (typically up to 5% by weight) of nano-sized particles of high aspect ratios ($L/h > 300$). This can lead to a radical modification in the host

polymer properties and performance. The composites developed so far include a variety of thermoplastic, thermoset and elastomer polymers, starch and biodegradable polyesters.[55] Nanocomposite films have also been developed for improving mechanical and barrier properties of plastic films or articles (containers) against gases and water vapour.[3,55]

Alumina, including synthesised wheel-shaped alumina platelets, can be used as fillers for plastic materials and are said to impart excellent mechanical properties.[4] Nano-precipitated calcium carbonate is claimed to improve not only the mechanical properties of polyethylene but also its heat resistance and printing quality – two properties important in blowing thin plastic films from molten polyethylene and then printing to form the final packaging.[5] Other forms of nano-sized synthetic calcium carbonate have been developed too, for improving the mechanical properties of composite plastic materials.[6] A nanoscale zinc sulfide has been used to give anti-ageing properties plus a synergistic effect with organic stabilisers to improve the durability of polymers.[7] However, food packaging has not been indicated as a potential application for this material.

6.3 Improvement of Barrier Properties

6.3.1 Nanocomposites

Polymer nanocomposites incorporating nanoclay exhibit good gas-barrier properties. They were among the first nanocomposites to emerge on the market as improved materials for packaging (including food packaging). Nanocomposites can also increase barrier properties against visible and UV light. Clay-based nanocomposites and their properties have been reviewed by Paul and Robeson.[8] The nanoclay mineral used most often is montmorillonite (also termed as bentonite), which is a natural clay obtained from volcanic ash and rocks. Nanoclay is relatively cheap and widely available. It is the most dominant commercial nanomaterial, accounting for nearly 70% of the total volume.[9] It has a nano-scaled layer structure that restricts the permeation of gases.

Substantial improvements in gas-barrier properties of polymer composites containing nanoclay, typically in concentrations between 2% and 5% have been claimed.[10,11] Such improvements have led to the development of nanoclay/polymer composites for potential use in a variety of food packaging applications, such as processed meats, cheese, confectionery, cereals, boil-in-the-bag foods, as well as in extrusion-coating applications for fruit juices and dairy products, or co-extrusion processes for the manufacture of bottles for beer and carbonated drinks (Figure 6.1).[12]

Optimisation of experimental variables involved in the elaboration of polypropylene and polyethylene films with nanoclay, to obtain films with good exfoliation, barrier and mechanical properties, were studied by Pereira *et al.*[13]

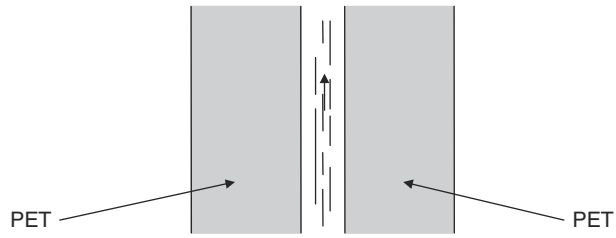

Figure 6.1 Typical sandwich structure of a polyamide containing a nanoclay, as a barrier layer in PET.

Nanoclay/polymer composites have been made from both thermoset and thermoplastic polymers.

Known applications of nanoclay in multilayer film packaging include beer bottles, carbonated drinks and thermoformed containers.[14] Examples of the currently available clay nanocomposites include Imperm® (by Nanocor and Mitsubishi Gas Chemical Alliance), and Aegis OX (Honeywell). Imperm® is used in multi-layer polyethylene terephthalate (PET) bottles and sheets for food and beverage packaging.[15] It works by minimising the loss of CO_2 from the drink and the ingress of O_2 into the bottle, thus extending the shelf-life by up to six months. Imperm is also used commercially in Europe in multi-layer PET bottles for beer and other beverages.[15] Duretham® LDPU 601 is a transparent plastic film with nylon which is enriched with silicate particles. Duretham KU 2-2601 uses Nanocor's clay to produce a film with increased barrier properties in the plastic, enhanced gloss and stiffness. The application is used where conventional nylon is too permeable and ethylene-vinyl alcohol coatings too expensive, e.g. paperboard juice containers.

Other additives used in polymer nanocomposites include polyhedral oligomeric silsesquioxane (POSS). The POSS-nanoclay is a relatively new hybrid based on the silsesquioxane-cage structures, one of the smallest forms of silica (also known as molecular silica), the physical forms of which can be liquid, wax or crystalline solid.[55] POSS-nylon composites with good barrier properties are used for food packaging applications.[9]

In addition to gas barriers, ENPs of metals and metal oxides can also be used to enhance light-barrier properties of the packaging materials. Additionally, ENPs have been incorporated as UV absorbers (e.g. titanium dioxide) to prevent photo-degradation of plastics such as PS, PE and PVC. The commercially important nanoparticulate materials in this respect are metals such as silver (Ag), zinc oxide (ZnO) and other oxides such as silica (SiO_2), titanium dioxide (TiO_2), alumina (Al_2O_3) and iron oxides (Fe_3O_4, Fe_2O_3). For instance, DuPont (Wilmington) has recently introduced a sun protection for plastic films and sheeting to protect plants in greenhouses or packaged goods from UV light. DuPont™ LightStabilizer 210 is an ultrafine/nano titanium dioxide (TiO_2) claimed to deliver broad-spectrum UV blocking and not to migrate out of the plastic under normal conditions.[16]

6.3.2 Nano-structured Coatings

Coatings are essentially continuous layers formed *in situ* or they can be comprised of individual nanoparticles held in a binder system.

Plastic films metalised with aluminium have been used as gas barriers and light barriers and as decorative films for decades. The aluminium layer is laid down by vacuum deposition techniques and is typically a few nanometers thick. Normally this metal layer is sandwiched in a multi-layer film construction, to prevent corrosion, scratching and abrasion that would spoil the optical (aesthetic) properties of the food pack. Other thin coatings on plastics include silicon dioxide (SiO_2), formed from the gases hexamethyldisiloxane and hexamethyldisilazane, which is used to make a plasma coating *in situ* on the inner surface of PET bottles and jars. The coating is intended to provide gas-barrier properties and the thickness is up to *ca.* 100 nm.

Nanoparticle-containing coatings with numerous nano-dispersed platelets per micron of coating thickness have been developed to increase barrier properties of PET to give longer shelf life of food and drinks.[55] The coatings are reported to be effective at keeping out oxygen and retaining carbon dioxide and can match other active packaging technologies such as oxygen scavengers. Examples include InMat Nanocoatings which is an aqueous-based nanocomposite barrier coating (possibly non-elastomeric) providing an oxygen barrier with 1–2 μm coating. Another example is silica/polymer hybrids manufactured by sol-gel process,[55] which can improve oxygen-diffusion barriers for plastics such as PET.

6.4 Improvement of the Performance of Bio-based Polymers

Bio-based polymers can be defined as polymers obtained directly from biomass (polysaccharides, proteins, peptides), polymers synthesised using bio-based monomers (e.g. polylactic acid), or polymers produced by microorganisms (e.g. polyhydroxybutyrate, bacterial cellulose, xanthan). Most bio-based polymers are also biodegradable. Typically, the use of biodegradable polymers as food packaging materials has so far been limited, because of inferior performance compared to synthetic plastics. These limitations include poor mechanical strength, high permeability to gases and especially water vapour, a low heat distortion temperature and poor resistance to protracted processing operations.[17] However, due to pressures set by environmental legislation, as well as by consumer demands, the interest in biodegradable polymers has increased in the last few years. This is also an emerging area of R&D with potential application of nanotechnology to improve properties of the biodegradable polymers. Potential perspectives of bio-nanocomposites in food packaging applications have been reviewed by Sorrentino.[17]

A typical example in this regard is that of polylactic acid (PLA). It is a biodegradable thermoplastic polyester that has a high mechanical strength, but

low thermal stability and low water vapour- and gas-barrier properties, compared to synthetic polyolefins (polyalkenes) and polyesters. Unmodified PLA is used in applications where these limitations are not critical, such as for yoghurt pots and as a water-resistant plastic layer in compostable paper cups for beverages. The incorporation of 5% (w/w) of montmorillonite into PLA has been reported to improve tensile modulus and yield strength along with a reduction in the oxygen permeability.[12]

Similarly, starch-based polymers have poor moisture-barrier and mechanical properties compared to synthetic plastic films. The incorporation of nanoclay in starch polymers has been reported to improve moisture-barrier and mechanical properties of biodegradable polymers,[56] as well as thermal stability and reduced water absorption of the composite system. Cyras *et al.*[18,] and Tang *et al.*[19] studied the effect of addition of nano-silica (SiO_2) to starch/polyvinyl alcohol films. They found that addition of nano-silica not only improved the material properties, but this also had no significant negative effect on the biodegradability of the films.

Mangiacapra *et al.*[20] prepared composites from pectin and two different montmorillonite clays by ball milling. They found an improvement in the elasticity of the film as well as in the water vapour- and oxygen-barrier properties.

6.5 Surface Biocides

Food contact materials with surface biocidal or biostatic properties should not be confused with active packaging (see Section 6.6). Surface biocides on packaging materials are not intended to have a preservative effect on the food. Instead, the biocidal agent is intended to help maintain the hygienic condition of the surface by preventing or reducing microbial growth and helping 'cleanability'. This being so, surface biocides are relevant to re-usable food packaging materials such as storage containers, transport crates, etc. Thus their relevance to single-use disposable packaging is questionable. Any release of the biocide from the surface of the packaging into the food can only be incidental, needs to be kept as low as possible and should not be at a high enough concentration to exert any preservative effect in the food. If there is any preservative effect in the packaged product, then additional regulatory authorisation as a direct food additive would be required in most countries.

The most common surface-active biocide is silver, used in a variety of chemical and physical forms including nano-silver. Silver has a broad spectrum of activity against both Gram-positive and Gram-negative microorganisms including the major food spoilage microorganisms. Among the commercial producers of nanomaterials with antimicrobial properties are Nanotech (Korea) with silver-based Sarpu.[21] Reduction of unwanted leaching of silver has been proposed by Johnston *et al.*,[22] who combined silver with nano-structured calcium silicate, hence tethering the Ag^+ ions to the large surface area of the nano-size platelets.

Nanotechnology has also opened a way for introducing other functionalities, such as antimicrobial activity to biodegradable materials. For instance, the preservative benzoic acid has been bonded to a magnesium–aluminium hydrotalcite and the complex has been blended with polycaprolactone to slow down the release of the antimicrobial molecule.[17] On the same theme, the EU-funded Solplas project (Functional nano-composite barrier coatings on plastic films via an aerosol assisted atmospheric plasma process) studied a combination of tailored chemical surface activation by cold atmospheric plasma treatment and subsequent wet-chemical coating using bio-based antimicrobial solution. This resulted in coated plastic films with good antimicrobial activity and improved barrier properties against oxygen transmission.[23] Other developments include the use of certain enzymes with antimicrobial activity, which could be covalently immobilised onto amino- or carboxyl-plasma activated bio-oriented polypropylene films via suitable coupling agents. Biomolecules attached onto the packaging surface retained the necessary enzymatic activity level to completely inhibit food spoiling bacteria in various conditions over a month-long storage.[24]

6.6 Active Packaging Materials

Active packaging is defined as a packaging actively improving and/or maintaining product quality. For this to be achieved, and to distinguish active materials from conventional 'passive' food packaging materials, there has to be some intentional material transfer between the packaging and the food. In this way, the packaging can be an absorber or a releaser of chemicals. Although there are a number of active packaging systems in prospect, so far relatively few use nanotechnology.

6.6.1 Nanoparticles in Oxygen Scavenging

Active oxygen scavengers can be based on metals such as iron or its lower oxides, which are oxidised and so consume oxygen under the appropriate humidity conditions. Another group of oxygen-scavenging chemicals are low molecular weight organic compounds such as ascorbic acid or sodium ascorbate. The third group of oxygen scavengers includes oxidisable polymeric resins together with a catalyst. Whereas the first and second groups (iron, ascorbate, etc.) are normally introduced into the packaging in sachet form, the oxidisable resins can be used to make the primary packaging as such. A wide variety of oxygen scavengers has been suggested, e.g. by Tammaji and Harish[25] in their patent application. Typically, an iron-based oxygen scavenger needs a large quantity of the active component. However, it has been proposed by Tammaji and Harish[25] that a combination of micrometer range and nanoparticulate iron enhances the oxygen absorption range due to increased surface area. Share and Pillage[26] also propose metal (cobalt) nanoparticle based oxygen scavenging material for potential use in packaging applications.

An example of a commercial active nanocomposite is Aegis® OX, a polymerised nanocomposite film, which is an oxygen-scavenging barrier resin formulated for use in co-injection PET bottle applications for oxygen-sensitive beverages including beer, fruit juice and certain soft drinks. The resins are a blend of active and passive nylon using O_2 scavengers and passive nanocomposite clay particles for providing barrier properties.

6.6.2 Nano-encapsulated Release Systems

Polymeric nanocomposites incorporating nano-encapsulated substances are polymers that incorporate nano-sized capsules containing different types of substances. They offer the possibility of controlled release of active ingredients into packaged foods. Current research is examining their potential uses in anti-bacterial packaging and for delivery of flavour and aromas. Many other applications for nanocapsules can be envisaged, as the substances that could be added to nanocapsules may include enzymes, catalysts, oils, flavour, colour enhancers, as well as nutritional compounds such as vitamins.[55]

6.6.3 Other Types of Active Materials

An interesting application for nano-structured calcium silicate is proposed by Johnston *et al.*[27] They suggest a composite material based on nano-silicate and a phase-change material, i.e. hydrocarbon wax, and show that the material can thermally buffer a paperboard package in varying temperature. The main application could be cold chain maintenance of a perishable food during transport and temporary storage during the distribution. The real-life performance compared to alternatives, such as simple insulation, is yet untested. The advantage of the use of a nanocomposite in this application is said to be the possibility to maintain the alkane in powdery solid form even above the melting point.

Dirt-repellent coatings have been developed by German researchers for packaging applications. The cleaning action is reported to be due to a 'Lotus Effect' – referring to the phenomenon that water beads and runs off the surface of lotus leaves due to nanoscale wax pyramids which coat the leaves. Certainly, water beads and runs off from any waxed surface (e.g. a polished car or waxed paper), but the exact advantage provided by such nanoscale pyramids is not yet clear. The proposed uses for the technology include self-cleaning surfaces that can help prevent growth of microorganisms and ensure food safety, such as in abattoirs and meat processing plants.[55]

Besides the immediate direct food packaging applications, Kuraray and Mitsui have described a conductive polyester fibre, made by absorbing carbon nanotubes on the fibre.[28] However, due to the current questions over the potential harmful health effects of carbon nanotubes,[29] the application of this antistatic material in food packaging is unlikely.

6.7 Intelligent Packaging Concepts

Smart or intelligent packaging is intended to monitor and provide information about the quality of the packaged food. The intelligent function can be based on the ability of a package to provide information about the requirements of the product quality such as package integrity (leak indicators) and time–temperature history of the product (time–temperature indicators). Smart packaging can also give information on product quality directly. For example, a freshness indicator can directly indicate the quality of the packaged product. Such indication is based on a reaction between the indicator and the chemicals produced either during oxidation (e.g. development of a stale and rancid off-taste), or during microbial spoilage. In broader sense, a smart package can also give information on the products' origin, physical handling of the package and whether the product has suffered tampering or pilferage.

6.7.1 Time Temperature Indicators

Visual time–temperature indicators (TTI) integrated into the packaging can indicate the correct maintenance of the cold chain. Several TTI systems based for instance on diffusion, enzymatic reaction, or polymerisation have been proposed and some of them have reached the commercial application stage. However, according to our knowledge, nanotechnology has not been used in this area so far.

6.7.2 Leakage Indicators

Since package head-space gas composition, especially the maintenance of low oxygen concentration, plays an important role in quality maintenance of many food products,[30] a lot of research effort has been directed towards leak indicators reacting to the ingress of oxygen. Several oxygen indicator compositions have been proposed.[31–34] In this kind of visual quality indicating system, nanotechnology can have a particular role, e.g. in the formulation of nanoparticulate-based printable inks. For instance, Mills and McGardy[35] have developed an oxygen-detecting ink containing light-sensitive nanoparticles, that are switchable by ultraviolet light.

6.7.3 Spoilage Indicators

Freshness indicators are intended to indicate directly the quality of the packaged product.[36] For example, a signal of microbiological quality could be a result of a reaction between the indicator and the metabolites produced during the growth of the microflora of the product. The capability of sulfur compounds to indicate the quality of packaged poultry meat has been exploited in a hydrogen sulfide indicating concept by UPM Raflatac. The indicator is based on a reaction between hydrogen sulfide and a thin layer of silver.[37,38] The thin silver layer starts as opaque light brown, but as silver sulfide is formed the layer

changes to transparent. The label can be used to evaluate product quality throughout the distribution chain and can be considered particularly useful at certain seasons, such as Christmas and Easter, which require the maintenance of large stocks of chilled poultry in the overflow stores.

6.8 Nanosensors for Food Quality

Further examples of complex nanotechnology-derived sensor systems that are currently under development include Nano Bioluminescence Detection Spray.[3,55] These nanosensors contain a luminescent protein of small molecular weight modified to bind to target microbial surfaces such as *Salmonella* and *E. coli*. When bound, the protein emits a visible glow thus allowing easy detection of contaminated foods and beverages. R&D into designing such spray techniques include spraying onto ocean freight containers in shipping of perishable goods and for defence and security applications in relation to bioterrorism. DNA-based biochips are also under development[55] that can detect pathogens and different kinds of harmful bacteria in meat or fish or the mycotoxin-producing fungi affecting fruit. Portable, micro-array based hand-held nanosensors for identification of pathogen contamination, harmful chemicals and toxins in food have also been developed in the EU-funded Good Food project.[3,55]

Another example is BioSilicon™ (from pSivida, Australia), which is nanostructured silicon with nanopores for potential applications in food packaging. The potential pSiNutria products being developed include products to detect pathogens in food, for food tracing, for food preservation, as well as products to detect variations of temperature in food storage. These products may include ingestible BioSilicon™, which will dissolve into silicic acid in the body.[39] Another relevant development is the Nano Bioswitch 'Release-on-Command' concept, which is aimed at providing a basis for intelligent preservative packaging technology that will release a preservative only when a packaged food begins to spoil.[57]

The incorporation of most of such active sensors into food packaging would, however, also require a power source. Among the potential power sources are biofuel cells and solar cells. Biofuel cells are devices capable of transforming chemical to electric energy via electrochemical reactions involving enzymatic catalysis. Various oxidoreductases can be applied as biocatalysts for the anodic or cathodic half cell reactions in biofuel cells, as recently reviewed by Minteer *et al.*[40] and Davis and Higson.[41] The enzymes enable operation of the cell using harmless chemicals (e.g. alcohol) as fuel under mild conditions. Biofuel cells can be utilised in various applications, including miniaturised electronic devices, self-powered sensors and portable electronics. If realised using roll-to-roll manufacturing methods (i.e. creating electronic devices on a roll of flexible plastic or metal foil), these bio-based devices could also be applicable in communicative packaging applications as disposable power sources. Many potential applications for these could be found, e.g. in active RFID (radio frequency identification devices), where a local power source enables longer reading distance and other

functions, such as memory and sensors. Our own studies (at VTT) have been aimed at the development of a cheap, disposable enzyme-based power source using biocatalytic layers.[42] The results so far have shown that the enzymatic activity can be retained and maintained for months in different conductive inks containing e.g. carbon nanotubes, depending on the storage conditions. Under optimised conditions, a fuel cell containing a laccase (oxidase)-based cathode maintained its capacity to generate power for several days.

Another example was provided by Strange *et al.*,[43] who described the solar cell devices prepared on polylactic acid (PLA) filled with nanoclay. Although acceptable performance could not be demonstrated, probably due to the inadequate dispersal of nanoclay, the work does indicate the trend and the potential for harnessing nanotechnology. The future developments in the field of nanotechnology can be expected to enable the incorporation of even more complex functionalities into food packaging. For instance, a printing method for 3D nanostructures of metal oxides has been developed.[44] Potential application areas for this may be seen in relation to food packaging, e.g. in regard to micro-fuel cells and gas sensors.

In general, the possibility to produce patterned conductive films, e.g. from silver nanoparticles, opens up possibilities for new intelligent and smart packaging concepts. R&D efforts have also been directed to the development of new sintering methods for nanoparticulate silver films. For instance, Albert *et al.*[45] propose a low-temperature, pressure-assisted sintering method for silver films produced using laser ablation of microparticle aerosol process. New conductive inks for ink jet printing based on stable copper ENPs, as cost-effective alternatives to silver and gold nanoparticle-based inks, have also been developed by Park *et al.*[46]

In the context of intelligent or smart nanotechnology based concepts, the combination of nanomaterials with biomolecules is likely to offer a range of new possibilities. For instance, Willner *et al.*[47] have described several examples of biomolecule–metallic nanoparticle hybrid systems. These concepts can be applied to various biosensing applications, but can also be used for the biocatalytic growth of nanowires and surfaces.

In addition to the microbiological quality of a food product, the safety of the product is also determined partly by its authenticity. Nano barcodes incorporated into printing inks or coatings have the potential to be used for tracing the origin of the packaged product.[48] So, for instance, Patel and Thanawala[49] propose combination of a water-soluble film and a security element, e.g. a nanoparticle-based barcode or a nano tracer, as a suitable security marker for packaging films.

6.9 Potential Migration of Nanoparticles from Food Contact Materials

All new packaging materials need to be evaluated for their safety and suitability for direct or indirect contact with food. The risk assessment of consumer

exposure to nanoparticles by the oral (food-borne) route is outside the scope of this chapter. The central relevant question asks if there is any migration of nanoparticles into food and, if so, how much (what concentration or number of ENPs) and of what type (size, shape etc) is there?

The establishment of guidelines for migration of ENPs from nanocomposites would be important. The need for such testing has been highlighted by a number of researchers. However, there are only two experimental studies to date. In the first, Avella *et al.*[50] measured the migration of the elements Fe, Mg and Si from a biodegradable starch/nanoclay nanocomposite film. Vegetable samples (lettuce and spinach) were placed into bags made of either potato starch, potato starch–polyester blend, and their respective composites with nanoclay. After storage for 10 days at 40 °C, the vegetables were acid-digested, and the migration of minerals determined by atomic absorption spectrometry. There was no increase in Fe and Mg in the vegetables compared to controls but there was an increase in Si – the main component of nanoclay. The concentrations of Si detected in the vegetables were 16–19 mg kg $^{-1}$ in the case of nanoclay composites of potato starch, and potato starch–polyester blend, compared to 13 mg kg $^{-1}$ for the same polymers without nanoclay, and around 3 mg kg $^{-1}$ in unpackaged vegetables.

A similar approach was taken by Bradley *et al.* (unpublished)[51] who studied migration from commercial beer bottles that had a nanoclay composite embedded between PET layers, and commercial polypropylene food containers containing nano-silver (Figure 6.2). In both cases, using ICP-MS analysis, there was no detectable migration of clay minerals. There was a very low silver migration, but the levels were less than the limit of quantification. Also, the

Figure 6.2 (a) Nanoclay containing PET bottles for beer. (b) Nano silver containing polypropylene storage containers.

presence of ENPs did not cause any significant changes in the migration of non-nano components from the two polymers tested.

Whilst it is the migration of nanoparticles *per se* that has attracted most concern to date, it must also be recognised that other chemicals are added too. In particular, nanoclays used as fillers are inorganic materials whereas the polymers into which they are incorporated are organic materials with a generally non-polar character. To make the two materials compatible it is usually necessary to modify the surface of the clay with organic chemicals. This is true also for conventional fillers such as glass fibres, titanium dioxide and silicon dioxide. However, because of the very large surface area of the ENPs, a far larger proportion of organic modifier has to be used and it may comprise up to 30% by weight of the ENP. If these organic chemicals remain firmly fixed to the ENP surface then any migration will be in proportion to the ENP migration itself. If, however, there are significant unreacted residues or any modifier not firmly attached, then a higher migration may occur.

Studies of the type described, measuring the migration of all chemical forms, allows an upper limit to be calculated for the migration of ENPs by assuming the worst-case of no migration of soluble and non-nano forms and so with the detection limit calculated for ENPs only. At present there is no alternative approach, since there are not methods available to test foods for low levels of ENPs.

6.10 Summary

In summary, nanotechnology products and applications can potentially revolutionise the food packaging sector, and meet many of the industry's needs in relation to innovative, strong, lightweight and active and intelligent materials. It also seems that, in public opinion, applications of nanotechnology in packaging is perceived as being more beneficial and less problematic than in other food applications.[52,53] A contributing factor to this seems to be the expectation that, due to the fixed or embedded nature of ENPs in plastic polymers, they are not likely to pose any significant risk to the consumer. A number of possibilities can be seen in the improvement of packaging material properties through the use of nanotechnologies, e.g. in the form of nanocomposites or nanocoatings, both for conventional plastic materials and biodegradable polymers. There are already examples where low levels of nanofillers have been used to increase the mechanical and gas- and light-barrier properties of food packaging materials. Additionally, nanotechnology can be used to introduce completely novel active functionalities to offer innovative packaging solutions. Nanotechnologies can also add other intelligent features to food packaging, e.g. in terms of monitoring the product quality through integrated sensors and indicators as well as establishing the authenticity of the products.

It is also imperative, however, that the development of such packaging innovations is steered carefully with all the safety considerations in respect to consumers and workers, as well as the environment. In this regard, the available

information is currently very sparse in terms of experimental data on the toxicology of ENPs that are (or can be) used to develop new food contact materials to provide a basis for adequate risk assessment. The risk is, however, dependent on both hazard and exposure – where the absence of one means no risk. Therefore, in the absence of hazard information, the focus is on the estimation of potential exposure. The results of migration testing (Bradley *et al.*, unpublished), and the estimates of potential migration through modeling[54] do provide some reassurance in the safety of nanotechnology-derived food contact materials. However, more testing on other types of materials is needed to build a broader picture in regard to the migration patterns for other nanocomposites that may be used in food packaging. Detailed toxicological studies are also needed to determine the potential effects of those ENPs that may get into food through packaging. These studies need to particularly focus on the health consequences of the long-term exposure to potentially low levels of ENPs via food and drinks.

References

1. Cientifica, *Nanotechnologies in Food Industry*, published August 2006, http://www.cientifica.eu/.
2. S. V. Lei, S. V. Hoa and M.-T. Ton-That, *Compos. Sci. Technol.*, 2006, **66**, 1274–1279.
3. T. Joseph, and M. Morrison, www.nanoforum.org, April 2006.
4. http://www.foodproductiondaily.com/news/ng.asp?n=78025-nanotechnology-plastics-molecules.
5. Additives for polymers, April 2008, 2–3.
6. Additives for polymers, 2006, 2.
7. Additives for polymers, 2007, 3.
8. D. R. Paul and L. M. Robeson, *Polymer*, 2008, **49**, 3187–3204.
9. Plastic additives & compounding, 2005, **7**, 18–21.
10. Z. Ke and B. Yongping, *Materials Lett.*, 2005, **59**, 3348–3351.
11. G. Qian, D. Jarus and V. Zabrocki, *PCT Patent* WO 2007/106671 A1.
12. Z. Akbari, T. Ghomashchi and A. Aroujalian, in the Food and Health Food Industries' Conference, organised by Institute of Nanotechnology, 25–26 October 2006, Amsterdam.
13. D. A. Pereira de Abreu, P. Paseiro Losada, I. Angulo and J. M. Cruz, *Eur. Polym. J.*, 2007, **43**, 2229–2243.
14. Plastic Technology, www.plastictechnology.com/articles/200508fa1.html.
15. Plastics Technology Newsletter, http://www.ptonline.com.
16. Additives for polymers, 2008, **1**, 5–6.
17. A. Sorrentino, G. Gorrasi and V. Vittoria, *Trends Food Sci. Technol.*, 2007, **18**, 84–95.
18. V. P. Cyras, L. B. Manfredi, M.-T. Ton-That and A. Vázquez, *Carbohydr. Polym.*, 2008, **73**, 55–63.
19. S. Tang, P. Zou, H. Xiong and H. Tang, *Carbohydr. Polym.*, 2008, **72**, 521–526.

20. P. Mangiacapra, G. Gorrasi, A. Sorrentino and V. Vittoria, *Carbohydr. Polym.*, 2006, **64**, 516–523.
21. *Plastic Addit. Compounding*, 2005, **7**, 13.
22. J. H. Johnston, T. Borrmann, D. Rankin, M. Cairns, J. E. Grindrod and A. Mcfarlane, *Curr. Appl. Phys.*, 2008, **8**, 504–507.
23. J. Vartiainen, M. Rättö, U. Tapper, S. Paulussen and E. Hurme, *Polym. Bull.*, 2005, **54**, 343–352.
24. J. Vartiainen, M. Rättö and S. Paulussen, *Packaging Technol. Sci.*, 2005, **18**, 243–251.
25. K. S. Tammaji B. Harish, *Patent Application*, US 2006/0069197 A1.
26. P. E. Share and K. R. Pillage, *Patent* US 7,244,484 B2.
27. J. H. Johnston, J. E. Grindrod, M. Dodds and K. Schimitschek, *Curr. Appl. Phys.*, 2008, **8**, 508–511.
28. *Nanomaterials News*, 2007, **3**(17), 2.
29. C. A. Poland, R. Duffin and I. Kinloch, *et al., Nature Nanotechnol.*, 2008, **3**, 423–428.
30. R. Ahvenainen and E. Hurme, *Food Addit. Contam.*, 1997, **14**, 753–763.
31. T. Mattila-Sandholm, R. Ahvenainen, E. Hurme and T. Järvi-Kääriäinen, *European Patent*, 0666977, 1998.
32. D. Perlman and H. Linschitz, *US Patent*, 4526752, 1985.
33. Y. Yoshikawa, T. Nawata, M. Goto and Y. Kondo, *US Patent*, 4349509, 1982.
34. R. Ahvenainen, T. Pullinen, E. Hurme, M. Smolander amd M. Siika-aho, *PCT International Patent Application*, WO 9821120, 1997.
35. A. Mills and M. J. McGrady, *Photochem. Photobiol., A*, 2008, **193**, 228–236.
36. M. Smolander, in *Novel Food Packaging Techniques,* ed. R. Ahvenainen, Woodhead Publishing Ltd, Cambridge, 2003, pp. 127–143.
37. M. Smolander, E. Hurme, M. Koivisto and S. Kivinen, *PCT International Patent Application*, WO 2004/102185, A1, 2004.
38. M. Smolander, in *Smart Packaging Technologies,* Wiley, Wiltshire, UK, 2008, pp. 111–127.
39. http://www.psivida.com/application/other.asp.
40. S. D. Minteer, B. Y. Liaw and M. J. Cooney, *Curr. Opin. Biotechnol.*, 2007, **18**, 228–234.
41. F. Davis and S. P. J. Higson, *Biosens. Bioelectron.*, 2007, **22**, 1224–1235.
42. M. Smolander, H. Boer, M. Valkiainen, R. Roozeman, M. Bergelin, J.-E. Eriksson, X.-C. Zhang, A. Koivula and L. Viikari, *Enzyme Microb. Technol.*, 2007, 2008, **43**, 93–102.
43. M. Strange, D. Plackett, M. Kaasgaard and F. C. Krebs, *Sol. Energy Mater. Sol. Cells*, 2008, 2008, **92**, 805–813.
44. *Nanomaterials News*, 2007, **3**(17), 6.
45. A. D. Albert, M. F. Becker, J. W. Keto and D. Kovar, *Acta Mater.*, 2008, **56**, 1820–1829.
46. B. K. Park, D. Kim, S. Jeong, J. Moon and J. S. Kim, *Thin Solid Films*, 2007, **515**, 7706–7711.

47. I. Willner, R. Baron and B. Willner, *Biosens. Bioelectron.*, 2007, **22**, 1841–1852.
48. M. Han, X. Gao, J. Z. Su and S. Nie, *Nat. Biotechnol.*, 2001, **19**, 631–635.
49. S. P. Patel and N. K. Thanawala, *PCT Patent*, WO 2007/015261, 2007.
50. M. Avella, J. J. De Vlieger, M. E. Errico, S. Fischer, P. Vacca and M. G. Volpe, *Food Chem.*, 2005, **93**, 467–474.
51. E. L. Bradley, L. Castle and Q. Chaudhry, Nanoparticles in food contact materials and articles, 2009, in preparation.
52. M. Siegrist, M.-E. Cousin, H. Kastenholz and A. Wiek, *Appetite*, 2007, **49**, 459–466.
53. M. Siegrist, N. Stampfli, H. Kastenholz and C. Keller, *Appetite*, 2008, 2008, **51**, 283–290.
54. P. Šimon, Q. Chaudhry and D. Bakoš, *J. Food Nutr. Res.*, 2008, **47**(3), 105–113.
55. A. Garland (ed.), *Nanotechnology in Plastics Packaging: Commercial Applications in Nanotechnology*, Pira International Limited, UK, 2004, 14–63.
56. M. Alexandra and P. Dubois, *Mater. Eng. Res.*, 2000, **28**, 1–63.
57. Advance Magazine, Feb 2004, www.azonano.com/details.asp Article ID-1317.

CHAPTER 7

Potential Benefits and Market Drivers for Nanotechnology Applications in the Food Sector

FRANS W. H. KAMPERS

Wageningen UR, P.O. Box 8026, 6700 EG, Wageningen, The Netherlands

7.1 Introduction

I like a juicy steak for dinner, accompanied by home-made French fries and completed with fresh lettuce and tomatoes, garnished with seasonal herbs. I prefer it to be lovingly prepared by a beautiful woman and together with her enjoyed in a candlelit ambience, with a good bottle of red wine, soft music, and a nice view of the sunset. Why in God's name would I need technology, and more specifically nanotechnology? What benefits does this technology bring to this table, why would anybody in his right mind eat something produced with advanced technology? Can it become any more artificial than 'Nano inside'?

To apply a new technology in any specific application field, there should be distinctive advantages. Every change comes with a cost and must be balanced by benefits to be accepted. This holds especially true for advanced technologies like micro- and nanotechnologies. Not only is there the factor of the investments that have to be made to accommodate the technology, there is also the uncertainty surrounding the introduction, caused by the risk that the customers do not perceive the benefits as the technology pushers did, or public perception turns against the technology, resulting in the failed introduction of new products and usually large losses. Although these aspects are valid for most

RSC Nanoscience & Nanotechnology No. 14
Nanotechnologies in Food
Edited by Qasim Chaudhry, Laurence Castle and Richard Watkins

Published by the Royal Society of Chemistry, www.rsc.org

application fields, they are especially applicable to the food sector. And even more so in combination with technologies like nanotechnologies, of which most consumers have very little notion, that intrinsically can not directly be seen to be present in the product and requires sophisticated instruments to detect it or to determine its benefits.

Not only is the food sector itself rather risk averse, so are the customers of the food sector. The consumers are highly conservative. It is therefore clear that, when most companies in the food industry have programmes to look into the new possibilities of nanotechnologies,[1] they must see very clear benefits and associated opportunities. In this chapter we will look at the different opportunities that can be identified. We will do that by taking the viewpoint from different levels of abstraction, starting globally and gradually descending towards the level of the individual consumer. Since nanotechnologies are very advanced and state-of-the-art technologies, we will look for desires and needs that are apparently not easily met by conventional agri-food technologies. In the end, we will hopefully have shown that nanotechnologies do bring new opportunities to the dinner table, that societies can get distinctive advantages, that industry can use them to innovate, and that consumers definitely can benefit from applications and in the near future probably will provide a 'license to produce', as described in Chapter 3.

7.2 Global Challenges

Since food and nutrition are basic needs for all living organisms, providing good food is a global issue. Everybody needs a daily portion of energy, and a fresh supply of building materials, to maintain both lifestyle and body. Of course, there are large cultural differences between food consumption of different ethnical groups and how to provide the organism with the nutrients it requires, but eating is universal enough to identify a number of challenges that are more or less common to all people on this planet, and this perspective looks like a good starting point for our quest for needs and wishes where nanotechnology can play a role in fulfilling.

7.2.1 Growing World Population

In the last decades of the twentieth century, it became clear that our need for growth, in every aspect of our lives, results in exponential growth curves. Exponential growth by definition is not sustainable. Our planet at some point in time will prove to be too small to sustain such a rate of growth. At that point in time, disaster strikes. If this pertains to things like 'playing golf' or even 'air traffic', consequences can be overcome. However, if we look at the primary needs of people, like food, things become more serious. Unfortunately, changing exponential growth trends, which are the cornerstones of our western economies, is not easy and, even if there could be mechanisms to control them, it would take decades to see the first effects. Mankind, therefore, must look at

all the options available to increase food production, in order to postpone the moment at which starvation will become the limiting mechanism. New technologies are one of these options and nanotechnologies can contribute to the efficiency of the global agri-food complex and primary food production.[2]

Measuring the environmental conditions locally provides valuable information to manage the crop and to apply water, nutrients or herbicides and pesticides at the right moment. This is one of the components of precision farming. At the moment, even measuring conditions like temperature, humidity, soil moisture, irradiation, etc. is not economically viable with sufficient spatial density. In the future, nanotechnologies will provide the so-called smart dust devices that can be distributed randomly over a field, create an autonomous sensor network and collect the desired data without additional actions from the farmer. The information is then used by the farm management system to take local action only when and where necessary, thus making an optimal use of resources like water, fertiliser and pesticides, and reducing the pre-harvest losses that are common to agriculture. Smart dust is an application which, at the moment, is being developed within the defence industry, but which in this way can also have peaceful applications. Only nanotechnologies can make smart dust devices cheap enough for agricultural applications.

Another opportunity nanotechnologies offer is to encapsulate herbicides and pesticides in active micro- and nano-containers. With nanotechnology, the shell of encapsulation systems can be designed in such a way that they are triggered to release the chemical only when the specific pest arises. This means that, after the product has been applied to the crop, an outbreak of a pest would be stopped immediately and would not spoil large parts of the area planted.

Also in animal husbandry, it is important to monitor the health and reproductive status of individual animals. It is obviously essential to maintain the health of individual animals to optimise the production, but also fertility is a crucial parameter to be able to inseminate at the right moment, to avoid that a reproductive cycle is skipped. At the moment, individual identification of animals becomes more and more common practice in industrialised countries. The systems used are often based on radio frequency identification (RFID) technology in which a passive transponder is attached to the animal and can be read at a small distance without line of sight. These transponders can also be injected subcutaneously. In that case, they can be combined with different sensors that provide information on the animal. Temperature is relatively simple, and already provides valuable information on the health and reproductive status; but also activity of the animal and hormone levels can be monitored in this way.

One of the benefits of electronic identification of individual animals is to be able to monitor transport of animals within a region. This is important at the outbreak of often highly contagious diseases. Being able to track and trace individual animals provides information so that these kinds of epidemics can be contained more effectively than is the common practice today. Only if the technology used is small enough, but especially cheap enough, to provide the information, these will become economically viable scenarios. Nanotechnologies will make important contributions here.

7.2.2 The Need for More Sustainable Production

Primary production of food uses a lot of the earth's natural, but scarce, resources like arable land and sweet water. Although plant production of food in principle is a closed CO_2 cycle, the transport and processing necessary to get it to the consumer as a food product is extensive and heavily relies on external sources of energy. This is not helped by the fact that modern consumers want to be able to eat virtually every product from every part of the world and all year around. We want it to be fresh, unpreserved (or only mildly) and it must be convenient for use. The result of these trends is that, of all the food produced in the industrialised countries, 30–40% is not eaten but is thrown away. Both from a humanitarian and a sustainability point of view this is highly undesirable and must change. It will require a change in attitude of consumers, but nanotechnologies can also help here. Food packaging materials can extend the shelf-life of products; sensing and diagnostic devices in the packaging can provide reliable information on the quality of the food product.

Another important aspect is the production of animal protein, which puts a lot of pressure on the ecosystem, even at a global level. Animal husbandry is a very inefficient system to produce proteins. Not only is the conversion rate from plant protein to animal protein very poor, it also requires large amounts of potable water as an input and produces a lot of waste. Moreover, animals are the predominant sources of NO_2 and CH_4, which are both much more potent greenhouse gases than CO_2 and therefore animal husbandry is a strong contributor to global warming. Eliminating the animal from the production of food protein sources would, therefore, make food production much more sustainable. Unfortunately meat is a complex foodstuff with highly desirable properties, and is not easily replaced by plant protein sources. Although it is not difficult to develop a meat replacement from other protein sources, to create a product that resembles meat and can appeal to meat consumers is not trivial. Nanotechnologies can be used to provide some of the solutions. Meat is a material with a structural hierarchy that provides the texture and the flavour of the food component. This hierarchy starts at the nanolevel with very specific protein structures and molecular assemblies, fibrils, muscle fibres, and in the end the muscle. A system to recreate the texture of meat, therefore, must start at the nanolevel and work its way up through micro- and meso-levels to the macrolevel of the meat product.[3] To design such a system will require intricate knowledge of and control at the molecular and supramolecular levels, so nanotechnologies and nanosciences (as discussed in Chapter 4) may help to realise it.

7.2.3 Food Safety

At the global level, food and waterborne diseases are still a big problem, especially in developing countries. Even in the industrialised world, many hospitalisations are linked to consumption of unsafe food, and constitute a large economic factor,[4] despite the fact that food in history never was as safe as

it is today. Providing safe food and clean water is a global priority that will benefit virtually everybody. Nanotechnologies can help in two ways to achieve this goal; it can be used to eliminate pathogens, and to monitor the quality of individual food products and signal if food safety is compromised.[5]

Nanotechnologies, but especially microtechnologies, can be used to separate certain food-borne pathogens from specific products. With microsieves, for instance, the dairy industry can filter out bacteria from raw milk. Not only is this a very simple way to pasteurise milk, it is also much more sustainable since it does not require extensive energy input to heat the milk. The system can only work because of the extreme uniformity of the holes in the microsieve, which makes sure that all of the bacteria are filtered out. The thinness of the membrane that results in very low pressure needed to get the milk through the sieve and back pulsing to remove the bacteria and maintain a good flow through the sieve are also essential. This principle is also being used to remove yeast cells from beer.

Naturally, this approach to get rid of pathogens can only be used in specific foodstuffs. For other products antimicrobial properties of certain nanoparticles can be used to reduce the microbial load inside a food package. The antimicrobial properties of silver are well known and have been exploited throughout the centuries. These properties are strongly enhanced in nano-silver particles since they have an extremely high surface-to-volume ratio. Nano-silver particles can be included cost effectively in certain packaging materials or in coatings of packaging materials to kill the organisms that come in contact with them. These packaging materials have been shown to extend the shelf-life of certain food products and to reduce quality deterioration because of spoilage.

Many packaged food products are thrown away because the 'use by' date has expired. Just using the time to estimate the quality status of the packaged product is very crude, since the quality also strongly depends on the storage conditions. The 'use by' date is often the result of worst-case scenarios regarding the storage conditions. With sensors in the headspace of packaged food products that can detect specific metabolic products that arise from the spoilage processes, a much more direct and accurate determination of the product quality can be made. This area of application is discussed in more detail in Chapter 6.

With diagnostic tests, the amount of spoilage organisms on a fresh product can be determined at an early stage in the food chain and, using models for the specific product that take various storage conditions into account, the resulting shelf-life of the product can be estimated very accurately. Not only will these strategies reduce the chances that people eat off-quality foods, they will also reduce the amount of good food that is thrown away because they are suspected of being spoiled. These types of sensors all rely on the use of nanotechnology to make them accurate, specific, sensitive, low cost, battery-free and reliable (see Chapter 6 for more details). If necessary, they can be combined with RFID systems printed on the package or included in the package to be able to read the information at different places along the chain and even at the consumer's home. In the not too distant future it will be possible that a domestic refrigerator will be able to warn the consumer that the milk is not good anymore.

7.2.4 Preventive Healthcare

At the moment, societies rely on curative healthcare to overcome illnesses and health problems. It simply means that an individual visits a doctor when he or she has developed a problem and assumes that the doctor will find a solution that cures it. Often medicines need to be applied, ingested, and in some cases a surgical operation becomes necessary to resolve the problem. This is a very costly way of providing healthcare and it has become apparent that this will not be a system that will be affordable for much longer, especially with increasing numbers of citizens who tend to live longer and the epidemic of welfare-related diseases like overweight and obesity. A paradigm shift is called for towards a new healthcare concept, in which the aim will be to try to avoid health problems from occurring instead of curing them afterwards. Food and nutrition are important components of this preventive healthcare concept.

The most basic function of food is to maintain the body and to provide it with the energy needed to function properly. The biochemistry of the body largely determines what nutrients are needed at what point in time. These needs in part depend upon the genetic make-up of the individual, which determines what proteins potentially can be produced by what cells, and for the other part are governed by the exterior circumstances that influence the processes in the body and determine which genes are switched on at a certain moment in time. Providing the biochemistry with all the nutrients in the right proportions is probably a good way to maintain health, apart from things like infectious diseases and accidents. However, it appears that food and nutrition is highly individual, and to fulfil it would require methods to determine the needs on a day-to-day basis. Fortunately, our bodies have alternatives for some of the nutrients, and have developed mechanisms to cope with shortages in certain periods in time. In fact, these mechanisms, which were very successful when man was a hunter/gatherer, now cause a lot of problems in terms of overweight and obesity. Living in perpetual abundance, our bodies have plenty of opportunity to store all the energy needed to overcome extended periods of shortage, which never come. Moreover, our need for energy has strongly decreased because of the lack of exercise and due to our well-heated environments and very effective clothing. In combination with diets that have a high energy content, it is easily understandable why the overweight epidemic is a global phenomenon.

The best solution to this problem is to eat and live more sensibly. A varied diet with two pieces of fruit and 200 g of vegetables a day provides everything a body needs and will ensure health. Unfortunately, there are very few people who (can) live and eat like that. Changing the behaviour of people or groups in society is very difficult.

To maintain a stable body weight, a person on average either needs to exercise much more and eat differently or much less. With our modern, high-energy diets, eating less in fact means eating very little. Unfortunately, that in turn means that we develop a shortage of certain nutrients. This problem especially occurs in certain groups in society that have very one-sided diets

(young people in certain cultural groups), or use very little energy (sick and elderly people). To maintain their health, these groups can benefit from food products that have been fortified with specific nutrients. Some nutrients, however, can not be added to all food products directly. In some cases these nutrients are broken down before they can be taken up by the body, some react with others, and for some the bioavailability is very poor. Other nutrients taste bad and would spoil the taste of the food product to which they are added. Nanotechnologies can be used to develop intricate encapsulation schemes to overcome these problems.[6] The encapsulation systems can be designed in ways that the nutrients are released in that part of the gastrointestinal tract where they are most effective. In this way, nanotechnologies can contribute to the preventive healthcare concept and will help maintain our society.

7.3 Food Industry Incentives

Industry, in general, is mostly interested in getting competitive advantages in order to secure financial success. The food industry is not much different, although in some areas craftsmanship and pride in the traditional product can also be observed. Whether or not the latter are derived from the first or vice versa is not always clear. One of the problems usually also observed in the adoption of revolutionary technologies like nanotechnologies by the industry is that the existing companies have invested in large installations to produce their products. Especially in food industry, the production processes are bulky and installations are designed to produce m^3 amounts, not cm^3. Adopting a new technology therefore often requires writing off substantial investments and abandoning production concepts that give them the competitive advantage, or at least of which their skilled personnel have intricate knowledge. That is the reason why it is often speculated that the companies that will be successful with a new technology are in the start-up phase at the moment. Nonetheless, large food companies all have research programmes that look at the opportunities that micro- and nano-technologies offer. And for some very good reasons.[7,8]

7.3.1 Product Innovation

Competitive advantage is gained by introducing products that do better than the ones that a competitor is selling. 'Doing better' in food products relates to properties that the consumer finds attractive. Often price is an important factor, but more and more aspects such as health, lifestyle and convenience are addressed. Advanced technologies usually are not well suited to reduce the price of food products. The product innovation opportunities that food industry looks for within nanotechnologies have to do more with the other aspects.

The encapsulation principles, which are a result of nanotechnology research, offer possibilities for new product characteristics. If healthy nutrients, e.g. omega-3 fatty acids, vitamins, minerals, etc., are included we enter the field of

novel foods.[6] Nanotechnologies are needed because they allow to design and develop specific functionality.[7] For clear beverages the 'containers' must be small enough not to scatter visible light. Some nutrients have more effect if delivered to specific parts of the gastrointestinal (GI) tract. Often the valuable nutrients are broken down by the food processing or in the first parts of the GI tract and need to be protected, or they must be kept apart from other chemical substances with which they would react if they came in contact.

Encapsulation also allows the design of new sensations in the mouth that could be introduced in new candy or drinks. The vesicle or micelle can contain substances that, when they come in contact with chemicals in the matrix or in another part of nanostructure, create sizzling sensations or a flavour boost, etc. It has been suggested that nanotechnologies allow for the design of food products of which the flavour can be chosen afterwards by the consumer. The idea would then be that several flavour compounds have been included in the product and with a specific trigger (e.g. certain microwave radiation) one of the components can be activated and the corresponding flavour can be experienced.

7.3.2 Process Improvement

Nanotechnologies, often in combination with microtechnologies, can be used to redesign processes in food industry for superior specifications or to develop new processes that allow new product concepts. Although food industry usually promotes a traditional or even artisan way of producing, most processes used are high tech and utilise modern technologies. Even in industries like wine, stainless steel and bulk processing have largely replaced the traditional barrels. The companies always look for opportunities to optimise the processes in regard to cost, product quality, efficiency, reproducibility and sustainability.

Emulsification is the work-horse of many food industries. Traditionally, an emulsion is made by high shear mixing of two substances that do not mix, in combination with surfactants that keep the small droplets of one of the components apart. With microtechnologies a totally new approach has been developed in which one component is pressed through a membrane into the second continuous component.[9,10] Although membrane emulsification as a principle was known in the pre-micro- and nanotechnology era, with the use of these technologies a new level of control has been achieved that allows emulsions with a very narrow droplet size distribution. The advantage of such a mono-disperse emulsion is that the stability is better. Having control over critical parameters up to the nanolevel also allows new concepts like double emulsions that can reduce the amount of the discontinuous component (e.g. oil in a mayonnaise) and replace it with a much cheaper or less calorie-rich component. In this way it can also be used as a delivery system for certain nutrients. In these processes, nanotechnologies can for instance be used to modify the surface of the processing device to improve the process

characteristics. By making the surface either hydrophilic or hydrophobic, very different product behaviour can be observed, and such modifications are often used to fine-tune the processes.

Although less suitable for large quantities, microfluidics also present new opportunities for the production of smaller quantities of food additives. In microfluidics, very narrow channels (in the order of magnitude of microns) are used. Fluids in these channels exhibit behaviour that is sometimes surprising and often can be exploited in certain processes. Because of the laminar flow, two fluids do not mix in the channel. This means that the mixing can be forced to take place at a very well defined point in the channel where the conditions are optimal. The small volume and the (relative) large surface area of the channel result in high temperature uniformity, which in turn results in a clean reaction without many side products. Also, if enzymes or catalysts are required in the production, a large surface to volume ratio is advantageous. Because of the small volume in the channel the process, even in the case of highly exothermic reactions, is intrinsically safe. This means reactions that for safety reasons would not be feasible in bulk can now be used. Of course, the amount of product that can be produced in a microfluidic device is very limited. But here maybe one of the biggest advantages, since these processes scale linearly. If the process is optimised in one device, producing 1000 times more simply requires 1000 devices in parallel, without the risks usually associated with scaling up to production volumes.

7.3.3 Product Quality

Never in history was food as safe as it is now. This is largely thanks to the high quality standards that have been implemented in the food industry. The quality of the end product is probably the single most critical parameter for a food company. In an industry that largely has to work with raw materials with variable composition and quality, this is a remarkable achievement. Moreover, every food product is susceptible to quality deterioration over time. The industry is well aware of its responsibility and vulnerability towards product quality and safety. It takes years to develop the image of high quality, but only one incident to destroy it. With the help of advanced technologies quality management can be improved.[5]

The key to product quality management is measuring critical parameters in the processes that are necessary to produce the product and deliver it to the consumer. Unfortunately most of the critical parameters can not be measured directly. When baking bread for instance, the quality of the end product strongly depends on the composition of the raw materials and the processes that take place in the oven. At the moment, it is not practically possible to determine the exact composition of the raw materials and the oven processes are simply managed by controlling the average temperature and the time. With nanotechnologies it will become feasible to measure certain critical parameters in the flour and yeast that allow fine-tuning of the process.

Moreover, it will be possible to measure certain volatile components in the baking process that directly correlate to the stage in which the process is at. These types of sensors will allow a greater control over the product quality, but will also make it possible to produce certain products with other production concepts.

For fresh products, quality assurance is even more critical. For these products, the time span between harvest and eating can be very short. Determining the amount of spoilage organisms on or in the product early in the production chain – together with models that can predict quality deterioration as a function of time and storage conditions – allows accurate predictions of the shelf-life of the product. Unfortunately, determining the cell count of spoilage organisms like *Salmonella* and others requires a well-equipped laboratory, highly skilled personnel and time. It often takes several days to incubate a sample and determine the average number of organisms. Fresh products can not wait that long to be delivered to the customer. With the help of the advances made in medical diagnostics, new tests based on nanotechnology principles are becoming available that allow much faster testing, closer to the production line, and by untrained production personnel. With these devices, quality assurance will reach the next level and can be incorporated in the logistic processes to deliver the right quality to the right customer at the right location.

While for spoilage bacteria the quantitative determination of the amount of organisms is called for, in the case of food-borne pathogens a more qualitative approach is sufficient. Pathogens are not allowed in food products at all, and regulation requires that a sample of 25 g is tested and not a single pathogenic organism is found. Apart from the fact that this is a totally different technological challenge, it also makes technologies that work with extremely small amounts less applicable. On the other hand, the molecular detection of chemical or biological signatures of these organisms is a perfect case for nanotechnology-based detection systems.

7.3.4 Extended Shelf-life

As soon as the raw materials for a food product have been harvested, processes start that change the quality of the end product. In only very few cases (e.g. wine) the quality improves with time, for other products deterioration takes place over time and in the fresh market this happens very rapidly. The food chain is always struggling with the short period in time after harvest in which the product has to be transported, processed, packaged, sometimes stored and delivered to the consumer. Logistic systems have been optimised to speed up the process as much as possible. However, if the shelf-life of a product could be extended, new opportunities for markets could arise and pressure on the supply chain would be reduced.

In the case of fresh produce, which is the most critical in this respect, spoilage by microorganisms is the process to be slowed down. Usually killing the

bacteria is an effective way to reduce their initial numbers. However, this is not an option for fresh products, since the consumer obviously wants them untreated. The best option for these products is to change the atmosphere in which the product is packaged in such a way that the organisms can not thrive, but the product is not changed. It usually means keeping out oxygen. Unfortunately consumers want to be able to see the product that they buy from the outside, which means that the packaging material must be transparent and most transparent materials have poor characteristics when it comes to stopping oxygen diffusion. In nanocomposites, nanoparticulate clay material has been embedded in the polymer matrix to improve the barrier properties of these materials.[11] It has now become possible to package beer, a product which is highly susceptible to oxidation, in PET bottles and maintain the quality for months.

As already discussed above, another way to improve the shelf-life of fresh food products is to incorporate antimicrobial properties in the packaging material. Silver nanoparticles exhibit antimicrobial behaviour. Incorporating nano-silver in the food packaging material can reduce the microbial pressure inside on the food product and thus extend the shelf-life. These aspects are discussed in detail in Chapter 6.

7.4 Consumer Benefits

The proof of the pudding is in the eating. And the eating in this case is literally done by the consumers. Whether or not advanced technologies will be accepted in food products strongly depends on the benefits that consumers perceive and whether or not they balance them with the risks, or, more accurately in the case of nanotechnologies, the hazards, that people see. It is, therefore, the case that the most important drivers for applications of nanotechnologies in food are to be found in the characteristics that consumers would like to see in food and can not easily be achieved with other technologies.

It is important to realise that consumers are not as rational as the technology developers would like them to be. Especially with food, a lot of emotional and even irrational behaviour can be observed. Discussing benefits from a rational point of view can sometimes have an adverse effect on the acceptance of the product. Moreover, risk/benefit evaluations are based on the assessment of usually very small chances for things to happen and people on average have a tendency to overestimate the implications of very small chances. The risk/benefit evaluation by consumers is usually dominated by emotions and 'gut feelings' on the one hand, and distrust and suspicion on the other. The unfamiliarity of large groups in society with nanotechnologies does not help to evaluate the risks and the benefits in a positive manner. These aspects have been discussed in detail elsewhere in this book (Chapters 2 and 3), but the main consumer aspirations that are likely to influence their choice of food products are summarised below.

7.4.1 Health

Food is one of the fundamental needs of the human body and with inappropriate food we can develop health problems. One of the basic functions of food as perceived by consumers therefore is to maintain the health of our body. Virtually everybody in the industrialised world is familiar with the idea that a varied and moderate diet with fruit and vegetables is good for health. Unfortunately very few adhere to these rules. Either because the rules are too cumbersome, or because we feel that they apply to everybody else but ourselves, or because we are healthy now and do not really believe that our health is threatened by our diet, and so forth. The bottom line is that most people know that there is a link between health and food, but still do not eat appropriately. Changing the behaviour of people is extremely difficult. We often rely on technology to compensate for our vices. If food products, especially if they agree with certain lifestyles, can be made healthier, that would be perceived as a definite benefit.

As has been explained before, one of the basic problems with food and health is that our diets contain much too much energy in relation to the needs of our modern lifestyles. If we want to maintain a stable body weight, we have to limit the amount of food that we eat. In effect that also reduces the amount of nutrients that enter our body. It can easily be that we develop a shortage of certain nutrients and are intrinsically malnutritioned with respect to these nutrients. It can be perceived for people with health problems, those who are hospitalised, or elderly people who require even less energy. Also certain subgroups in society with very one-sided diets are vulnerable.

As has been discussed before, nanotechnologies can be used to fortify food products with specific nutrients to make them healthier. In principle, it is possible to add some of the good qualities of milk, for example, to fizzy drinks or to eliminate part of the calorific value of mayonnaise by replacing most of the oil with water in a double emulsion (Chapter 5). It often requires nanotechnologies and intricate encapsulation systems to realise the functionality that allows maximum benefit from the added ingredients.[8] When the taste of certain nutrients does not agree with the taste of the product, stealth is necessary to 'smuggle' them through the mouth and into the GI tract. Often certain nutrients have poor bioavailability and, since the uptake of the substance takes place at the surface of the droplet or particle, the surface to volume ratio must be kept high. In some cases the nutrients must be delivered to certain parts of the GI tract for maximum effect, etc.

7.4.2 Cost

Although we spend a fortune on medicines and medical treatment if we have a health problem, we are usually not inclined to spend very much on food that will maintain our health. Margins in food are low and consumers often do not want to pay more for food products that claim to be healthier. This means that there is very little economic room to apply high-tech systems in food. In effect,

product innovations in the food industry preferably should have definitive benefits *and* be cheaper than the competing products. Of course the already discussed benefits like improved processes in the food industry, increased shelf-life of fresh products and less good products discarded will have a positive effect on the price of food. However, to develop new functionality without increasing the price of the end product is a challenge. Understanding the behaviour of matter at the nanoscale can provide tools that can provide a solution to such challenges. It was already recognized from the outset that it would be impossible to produce nanotechnology components in the classical, one by one, way. Because of the small sizes, it would need enormous numbers to build macroscale products. Drexler therefore proposed the concept of 'self-replication' (nanotechnology devices can duplicate themselves) and 'universal assembly' (a device can build large amounts of nanotechnology devices)[12] as a solution. Although these concepts have not been implemented, nanotechnology has provided us with the knowledge to design systems that can self-assemble. Molecules with very specific shapes and charge distributions under the right circumstances spontaneously form superstructures that, when designed well, create the desired functionality. Nature in fact has shown us the way, since all biological systems are the product of self-assembly. This concept provides a route to cheap production of new functionality at the nanolevel. Since it does not need much energy or mechanical inputs after the molecules have been formed, it can often compete with traditional ways of processing in the food industry and can result in lower production costs for these new products. Unfortunately, food applications are very limited in the molecules that can be used, keeping in view that food products must be safe for the consumer. The materials that are allowed must either have been extensively tested, which is very costly; have been used already in food applications; or are classified as 'Generally Regarded As Safe' (GRAS). This means that the library of molecules to choose from is small, and if new molecules are introduced they need to go through expensive testing for toxicity and long-term effects. This seriously limits the possibilities of self-assembly and as a result also limits cost-effective innovations in food. The library of molecules can be expected to expand over time, creating new opportunities as the technology matures and the general public is more accustomed to various applications of nanotechnologies in different areas.

7.4.3 Tasty

Eating, for a large part, involves enjoying the flavour, taste and texture. In fact, evolution has taught us to like those products that provide important nutrients (sweet for energy; salt for minerals). Our sweet tooth is directly related to the need of early humans to get as much high calorie food (e.g. ripe fruits) as possible when it was available, to be able to overcome the periods of scarcity. Unfortunately, at the moment, the opposite is the case in modern societies: food is abundant and always available. Moreover, since we still have a liking

for sweet products, the amount of sugar has increased gradually in our diets. The challenge for the food industry is to provide products that appeal to our tastes but contain fewer calories. In Section 7.3.2 the example has been discussed of double emulsions to replace substantial amounts of oil in mayonnaise. Sugar replacements are well-known examples of this trend. A difficult challenge is the reduction of salt in food products. With encapsulation mechanisms, tools are being developed to provide new options. Taste is experienced in the nose and taste buds in the mouth, whereas the calorific value of the food is extracted in the lower parts of the GI tract. In fact when the food has passed the mouth there is no further need for the taste components. All the sugars and salts that have not triggered a taste response are largely superfluous.

Releasing the molecules that stimulate the smell and taste receptors in nose and mouth is a surface process, if they are in the solid phase. Increasing the surface to volume ratio in smaller particles will create a strong taste experience with less of the substance in the product. Moreover, with intricate encapsulation and delivery systems, it is possible to release certain components in the mouth where the taste can be experienced. In this way, it will be possible to develop products that have at least as strong a taste experience as traditional products, but with much less of sugar or salt components.

7.4.4 Convenience

There is a strong trend that consumers do not want to spend much time getting food or preparing meals. Convenience is one of the high priorities for the general public. More lettuce is being sold pre-cut than the traditional cabbage, although it requires packaging and spoilage is enhanced. Microwaves are one of the biggest and most successful innovations in our kitchens because of the convenience they offer.

Most of the convenience that is found in food products comes from the fact that part of the preparation has been done by the manufacturer. This is only possible if the packaging is effective. In fact the convenience is often derived or made possible by the packaging concept.

In principle, it is possible to develop food products that contain particles that pack various flavour components. The associated flavours would only be experienced if a component is activated during the preparation. In this way it would be possible to buy the same product a number of times and each time it is consumed it could have a different taste, tailored to the preference of the particular consumer at that moment.

7.4.5 Lifestyle

Sometimes trends in food consumption develop within certain cultural groups that predominantly do not bear on the nutritional value or even taste, but because they become ‘hip’ or are part of a lifestyle. Maybe the best example of such a trend is alcohol. For the food industry these trends are very interesting,

because they usually give a strong boost to the turnover of the companies that sells the product, whereas the competing products lag far behind. Product innovations that create new sensations are therefore always interesting, since they potentially could create a hype. Apparently these products do not predominantly rely on taste or nutritional value to be appealing to the consumer. Their texture is different or they create a mouth feel that is unexpected. Nanotechnologies, with their toolkit of encapsulation and texture modification tools, can provide new opportunities to create these types of products.

7.4.6 Fresh

Most people prefer their food products fresh or only mildly preserved. Since we need not rely on harsh preservation methods to ensure the safety of our food, the trend has set in to eat as fresh as possible. But freshness, convenience and quality are often mutually exclusive properties for a food product. As discussed before, nanotechnologies can improve the barrier properties of packaging materials so that they are better suited to maintain the freshness of food products, even after they have been washed and cut for the convenience of the customer. In combination with indicators or sensors monitoring the headspace of the product, real information about the freshness can be obtained. These solutions can prevent good products being unnecessarily thrown away that do not look good, and products being consumed that look perfectly good but have high levels of pathogens.

Freshness of food products is a compromise between the distance between the producer and the consumer. By measuring the amount of spoilage organisms in the product directly after harvest, in combination with quality assurance models that take the storage conditions into account, good predictions can be made about the freshness of the products and decisions can be made which products to ship where and by what time they should be in the supermarket. Devices based on nanotechnology-based sensors, in combination with RFID technology, as discussed before, can continuously monitor the freshness of the produce and logistics can be fine-tuned to get the right freshness of product to the right customer.

7.4.7 Natural

Although a highly subjective and emotional aspect, ‘naturalness’ scores high in the ranking of preferred properties of food. How natural is wine or beer or Coke? How would we get cheese without the use of biotechnology? Where do candy bars grow? Still, consumers would like to have a perception of natural when they buy or use a food product. Technologies, especially high-tech ones, evoke the opposite perception: they are unnatural. As a matter of fact, much of the perceived hazards can directly be related to this perception. It is one of the reasons why the food industry is reluctant to show how they produce their products, and invariably show green pastures and natural environments in

commercials for their products. Of course there is very little that nanotechnologies can do against this common perception. Even in those cases where nanotechnology can reduce processing or actually make food more natural, the general public for the time being will regard it as unnatural. The products that rely on the new opportunities that nanotechnologies offer probably should not be advertised as natural but should derive their unique selling points from other properties. It could be counterproductive for the food industry to rely on the strategy not to disclose the use of these types of advanced technologies and to promote their products as natural. This course of action probably at some point in time will backfire, although some successful food industries have managed to maintain the image of natural, while in fact they heavily rely on biotechnology or other high-tech systems.

7.4.8 Tailored to the Individual

The success of Senseo coffee machines in some countries have shown that there are commercial possibilities to bring the final processing of certain products closer to the consumer. Mixing your own blend of flavours for a specific product in the supermarket, or even at home, to tailor the taste to your individual preferences will be possible in the near future. One of the difficulties in these concepts is that flavours are usually vulnerable molecules that easily oxidise or are broken down when they are outside their own matrix. For these concepts to work, the different flavour components need to be protected against breakdown. Again nanotechnologies with encapsulation of the flavour substances can be called for here. The encapsulation systems can be designed in such a way that the flavours are preserved under storage conditions, but are released by an external trigger, like heat or mechanical force (stirring). Also the presence of other molecules or the bulk matrix of the food product can make the encapsulate to break apart and release its contents.

7.5 Discussion

From the above it is clear that there are many benefits to be had from nanotechnologies for various parties involved in producing and consuming foods or the results of that. Some of these applications are easily acceptable, others are more controversial. A key element is the risks perceived. The risks of novel sensors and innovations in the processes to produce food products are small, whereas the benefits can be large. These applications of micro- and nanotechnologies are therefore more acceptable than the product innovations where the technology is part of the foodstuff and ends up in the body. These mostly are the encapsulation systems that can deliver essential nutrients to the body. They are, however, designed to break up and consequently will not persist in the body. Unfortunately these aspects do not seem to change the perception of consumers on the possible risks of these applications.

It is speculated that the term 'nanotechnology' is bound to raise negative perceptions, because it generates associations with biotechnology and other technologies that are commonly regarded as unnatural and risk prone. This is one of the reasons that the food industry refrains from using the term 'nanotechnology'. Communication about the different types of nanotechnology and the distinction between nanotechnology and nanoparticles will eventually change the perception of the relatively harmless applications in food. The lack of openness on the industry's part is counterproductive for the communication and could seriously backfire in the case of an incident. Trust can be gained by communicating about the new products from the perspective of the consumer and addressing their worries about the perceived risks.

To mutually share the benefits of the new technological developments, consumers need to develop trust in the applications of nanotechnologies in food. In addition to the communication, appropriate regulation can also contribute towards building of trust. Regulation implies that an impartial body, often a government body, has evaluated a particular application and has verified that its use in food is safe. To benefit most from this effect of regulation, it is necessary that the presence of regulation is communicated. In food applications, this usually implies labelling. Once approved for use in food, a nanotechnology application could be given a code with which informed consumers can retrieve information on the risks and benefits of the specific component in the product. However, the lack of clear definitions, complexity of the subject and the problem that nanotechnology is virtually undetectable in most foodstuffs makes the risk governance of nanotechnology in food very difficult.[13]

Getting a 'licence to produce' from the consumers for food products that use high-tech solutions to create specific benefits is the only way to introduce new products on the market. This is a generally applicable fact, but is more specifically valid in the food market. Food is something people take inside of themselves. Moreover, once eaten, it can not be removed from the body easily. That is why consumers are very particular about the things they eat. Prehistoric man already learned to be conservative and be cautious about new foods. New foods had to be tried and tested over long periods of time to determine their safety and nutritional value before they were accepted as routine food items. This is still one of the driving mechanisms for the conservatism of consumers towards new food products and the application of technologies they can not detect in foods. It is, therefore, of utmost importance that new nanotechnological developments are primarily aimed at addressing the needs and aspirations of the consumers, and are not seen as benefiting the industry alone.

References

1. Institute of Food Science & Technology, Trust Fund: Information Statement, *Nanotechnology*, 2006.
2. Nanoforum.org, *Nanotechnol. Agric. Food*, May 2006.

3. J. M. Manski, A. J. van der Goot and R. M. Boom, *Biomacromolecules*, 2007, **8**(4), 1271–1279.
4. WHO Global Strategy for Food Safety, 2002.
5. E. Kress-Rogers and C. J. B. Brimelow (eds.), *Instrumentation and Sensors for the Food Industry*, Woodhead Publishing Limited, 2nd edn., 2001 (ISBN 1 85573 560 1).
6. H. Chen, W. Jochen and S. Fereidoon, *Food Technol.*, 2006, **3**, 30.
7. C. I. Moraru, C. P. Panchapakesan, Q. Huang, P. Takhistov, S. Liu and J. L. Kokini, *Food Technol.*, 2003, **57**, 24.
8. J. Weiss, P. Takhistov and D. J. McClements, *J. Food Sci.*, 2006, **71**, 107.
9. C. J. M. van Rijn, *Nano and Micro Engineered Membrane Technology,* Elsevier, 2004.
10. P. Kumar and K. L. Mittal (eds.), *Handbook of Microemulsion Science and Technology*, ISBN 0824719794.
11. S. Moore, *Modern Plastics*, 1999, **76**, 31.
12. K. E. Drexler, *Engines of Creation*, ISBN 0-385-19973-2.
13. A. Grobe, O. Renn and A. Jaeger, *Risk Governance of Nanotechnology Applications in Food and Cosmetics*, Report from IRGC (2008), ISBN 978-2-9700631-4-8.

CHAPTER 8

Engineered Nanoparticles and Food: An Assessment of Exposure and Hazard

LANG TRAN[a] AND QASIM CHAUDHRY[b]

[a] Institute of Occupational Medicine, Research Avenue North, Riccarton, Edinburgh EH14 4AP, UK; [b] The Food and Environment Research Agency, Sand Hutton, York YO41 1LZ, UK

8.1 Background

Nanotechnology is one of the key emerging industries worldwide. Like every new technology, nanotechnology brings with it new benefits as well as potential new risks. Of paramount concern in this regard are the potential health risks to workers and consumers that might arise from exposure to nanomaterials, especially the free engineered nanoparticles (ENPs), either at work or through consumer products. These risks, if not assessed and managed properly, could not only jeopardise the growth and development in this area, but more importantly, could also have grave consequences for human health and the environment.[1,2] The development of safe nanotechnology products is, therefore, essential to establish and sustain the trust and confidence of the end users. Consumer confidence and acceptance of the new technology are the ultimate guarantees for the sustainable future growth in the vast range of nanotechnology enabled sectors. It is, therefore, essential to develop an effective approach for improving the assessment and management of potential consumer exposure to ENPs.[3]

RSC Nanoscience & Nanotechnology No. 14
Nanotechnologies in Food
Edited by Qasim Chaudhry, Laurence Castle and Richard Watkins

Published by the Royal Society of Chemistry, www.rsc.org

Nanotechnology has the potential to impact many aspects of the food and agricultural systems. These encompass new developments in the form of innovative packaging materials, novel (health)food products, nutrient delivery systems, enhanced bioavailability of nutrients and supplements, new tools for molecular and cellular biology and new materials for pathogen detection.[4 6] Food products containing nanotechnologies have already started to appear on the markets worldwide, albeit predominantly outside the EU at the moment. As with any new and evolving technology, much of the current emphasis is on the potential benefits of nanotechnologies for the industry and the consumer, and very little is known in terms of safety aspects of many of the known and projected applications in food.[6,7] The rapid emergence of nanotechnology has also led to demands for swift action by policymakers to ensure that the health and environmental risks are adequately assessed and regulated. As many nanotechnology derived (health)food products are already on the market, and the uncertainty about the potential risks is large, the need for science-based adaptation of the regulatory frameworks is also high.[7]

The potential hazard that ENPs may pose to human health and the environment is now a well-recognised issue.[8] Humans can be exposed to ENPs accidentally in the workplace, or through the use of a variety of consumer products (e.g. surface coatings, skin lotions, food, food packaging, etc.). ENP exposure is, therefore, possible via different portals of entry such as the lung, the gut and the skin. The study of ENP toxicity is now the topic of a new discipline – nanotoxicology. Much of the current toxicological data on particulate materials, however, relates to larger particles. ENPs, on the other hand, have certain distinct properties that may lead to altered biological interactions compared to larger sized or bulk counterparts, and the need for further investigations of these has been highlighted in a number of recent reviews and reports.[6 7,9] Currently, there are a number of knowledge gaps in relation to the hazards that ENPs in (health)food products might pose to the consumer's health. The major focus, however, is on two aspects – toxicokinetics and dose–response relationships. Toxicokinetics of ENPs is of special interest because the small size of free ENPs enables translocation beyond the portal of entry, to the blood system, from where they can reach other body organs.[10] This presents a new toxicological paradigm, as such organs are normally protected against the entry of larger particulate materials. This also makes it essential that dose–response relationships for ENPs are determined in relation to the potential affects on multiple organs. The potential effects of ENPs may therefore extend beyond the pulmonary system, which has so far been the focus of nanotoxicology research due to the studies on airborne ENPs in occupational and consumer environments.[11] Other organ systems, such as the central nervous and the gastrointestinal (GI) systems, are now coming under the main focus of nanotoxicology research.

The current toxicological endpoints of interest in relation to ENP exposure include oxidative stress, inflammation and genotoxicity. However, to assess the health risks from exposure to ENPs, the potential routes of exposure need considering, which poses a major challenge in view of the current scarcity of the

available information. The exposure assessment for an ENP-containing product, therefore, needs to take into account different exposure scenarios, as well as the frequency, extent and duration of the likely exposures.

8.2 Sources of Exposure

Materials derived from nanotechnologies are increasingly used by the (health)food sector for consumer products. This use is expected to grow considerably in the near future.[12,13] The current applications range from the improvement of taste, flavour and colour of food products, to enhancement of bioavailability of nutrients, development of active food packaging materials and nanosensors for monitoring the quality of food products during storage or transportation. The research carried out by the pharmaceutical, nutraceutical and cosmetics sectors into the ability of ENPs to interact with biological materials at the near-molecular level is also attractive to the food industry. There are examples of joint-development of cosmetic-nutritional supplements by the food and cosmetic companies.[13]

This chapter will look into the available information in regard to potential hazards that ENPs in food may pose to a consumer. Due to the scarcity of the available data on ENP hazard, the emphasis will also be to identify the route(s) and likely extent of the consumer exposure.

8.2.1 Types of Nanoparticles Used in the Food Production Chain

As discussed elsewhere in this book (Chapters 1 and 5), the types of ENP used in the food include both organic materials, such as nano forms of certain additives, and nanomicelles for delivering nutrients and supplements, as well as inorganic materials, such as silver, silicon dioxide, magnesium oxide, titanium dioxide. Examples of the ENPs used in food packaging include silver (Ag), zinc oxide (ZnO), silicon dioxide (SiO_2) and nanoclay.[6]

Some ENPs are already being used as replacements of bulk counterparts in food additives, and such applications are currently being subjected to restrictions only in a few countries. For example, aluminium oxide, lanthanum particles and nanoscale iron powder are used in the process of water purification and/or soil remediation. Silicate ENPs, and polymer nanocomposite with silver, magnesium and zinc oxide are used in food packaging materials. Inert ENPs are also processed in food products, examples of which include titanium dioxide, calcium, magnesium and silica. One of the main benefits claimed for nano-sizing the particles for use in (health)food applications is that this increases the bioavailability of the compounds. In this regard, it is important to note that the physicochemical properties, behaviour and effects of most of these ENPs are unknown. Consumer exposure can be expected following either direct ingestion of the particles via food and drinks, or through migration of the particles from packaging materials to the packaged food. The latter form of

exposure can be expected to be low, as long as ENPs remain fixed or bound in the packaging materials or in the coating on surfaces of packaging materials and food preparation devices.

It is evident form the known and projected uses of nanomaterials in (health)food products[6] that they can be broadly classified on the basis of material types and known physicochemical properties. A conceptual categorisation of the risk potential of different nanostructures and nano-sized additives is presented in Figure 8.1. In this regard, there needs to be a clear differentiation between the natural or processed nanostructures and nano-additives in food that are soluble or can be assimilated and/or excreted from the body, and those that are insoluble, indigestible and bio-persistent and can potentially translocate from the GI tract to other parts of the body. Whilst the former type of nanomaterials is not likely to behave any differently from the conventional equivalents and may not be of concern in relation to consumer safety, the use of the latter type in food may raise concerns over the potential harmful effects on consumer health. Concerns have also been raised in regard to nanodelivery systems,[14,15] which are generally built from peptide or lipid monomers.[4,16,17] Applications of such nanoencapsule-based delivery systems span from R&D into pesticide formulations (e.g. increased crop adherence and delivery to the pests), to a number of available products that are based on delivery systems for bioactive compounds. Such novel formulations may lead to an increased human exposure as a result of residues in the foodstuffs, or because of the increased bioavailability of certain additives, such as food preservatives.[6]

8.1.2 Characterisation of ENPs in Foodstuffs

To assess which physicochemical properties of ENP are responsible for the adverse health effects is one of the major issues being addressed by research under nanotoxicology. The current research efforts in this field have highlighted that ENPs can potentially undergo complex interactions in biological environments. In particular, ENPs can bind different biomolecules, such as proteins, on the surfaces. This surface coating can alter the ENP characteristics, their translocation and distribution, and cellular/molecular responses in the body.

The identification and validation of ENPs in foods requires not only the identification of individual particles but also determination of their disposition within the food matrix. This is because their toxicity depends on their state of aggregation and the component within which they are found. For example, aggregated nanoparticles held within an insoluble and indigestible matrix will pass through the body without being absorbed. On the other hand, individual nanoparticles separated within a lipid dispersion (orange oil emulsion in an orange drink for example) are much more likely to be transported across the endothelial layer in the intestinal lumen and into the bloodstream.

The ability to measure the internal dose of an ENP is essential in the construction of an appropriate dose–response relationship. At the moment, these

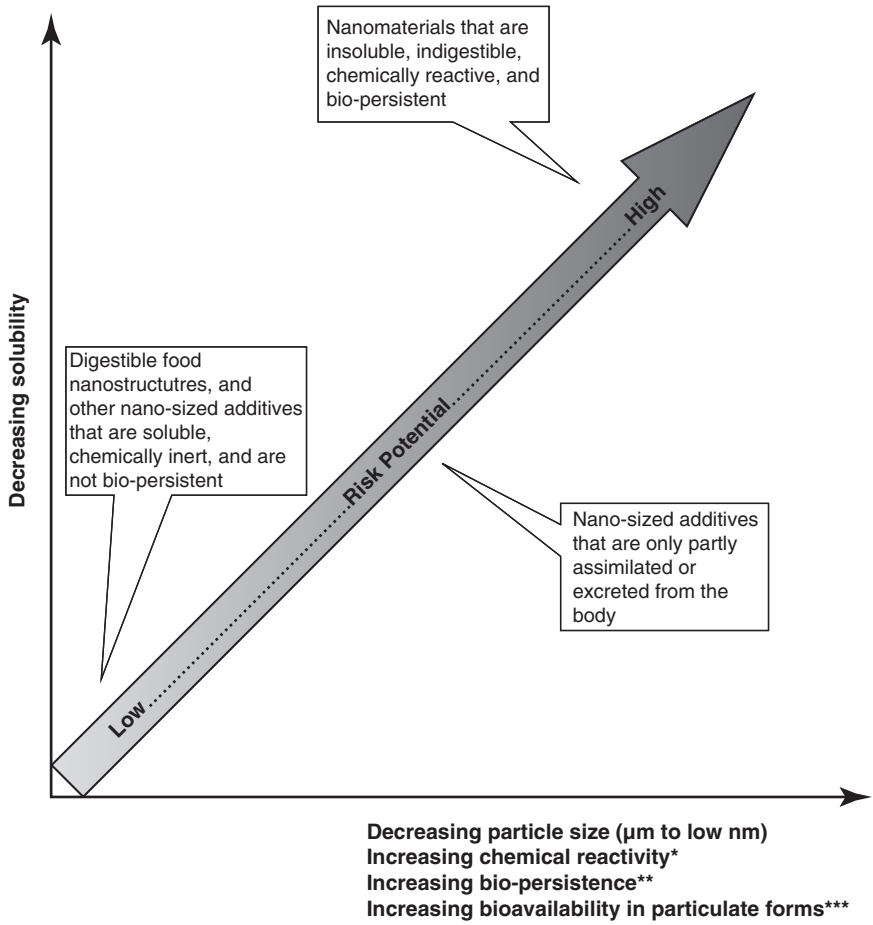

Figure 8.1 Conceptual categorisation of nanomaterials relevant to applications in food and food packaging. *inherently reactive, or surface functionalised materials **where the nanomaterials are not assimilated in the gut or at cellular level, or excreted from the body ***will be dependent on a number of factors, such as particle charge, surface coating, etc. The toxico logical hazard of any nanomaterial may be higher if the chemical con stituting the nanomaterial is toxic in nature, e.g. a pesticide or a toxic heavy metal.

methods are preliminary, and there is still a need for further development and validation. In regard to characterisation of the ENP added to a food matrix, there are currently very few established methods and procedures in the peer-reviewed literature. The complexity of the food matrix, the wide variety of the applied and proposed ENP types, and the relatively low concentrations of ENPs in final food and beverage products make their determination in real food samples very difficult.[18]

8.2.3 Route of Exposure

The main likely route of entry of micro- or nano-sized particles to the gut is through the consumption of food and drinks, although some entry through clearance of lungs is also known to take place. The consumer safety implications from the consumption of nanotechnology derived foods are, therefore, intrinsically linked to the physicochemical properties of the ENPs ingested, and the likelihood, extent and frequency of the exposures. It is known that ENPs have much larger surface areas and they may also exhibit substantially different physicochemical and biological properties compared to conventional bulk equivalents. So far, very few studies have been carried out into the toxicology of nanomaterials following oral intake, and much of the published research relates to either *in vitro* studies in cell cultures, or *in vivo* studies relating to inhalation exposure to ENPs. Therefore, there are major knowledge gaps in relation to the behaviour, interactions, fate and effects of ENPs inside and outside the GI tract. An important aspect in this regard is that the ENPs added to foods are likely to undergo a variety of transformations in food, and/or in the GI tract. For example, due to the enormous surface energies, most ENPs are likely to agglomerate, bind with other food components, or transform or degrade upon reaction with stomach acid or digestive enzymes, and therefore are not available in free particulate forms for translocation across the GI tract. Any significant risk of consumer exposure will thus be expected only if the ENPs added to food remain in free and insoluble nanoparticulate forms, or if they become available in such a form for translocation at any stage during the GI passage.

A healthy digestive system allows absorption of nutrients from the gut only when food components have been broken down to smaller units during digestion. The gut wall is designed to allow passage of dietary nutrients, but to prevent larger (insoluble) particulates or foreign materials getting into the blood circulation system. As nano-sized food ingredients and additives are likely to have a greater ability to cross the gut wall compared to conventional bulk equivalents, their enhanced absorption and bioavailability would give rise to a greater internal exposure. Furthermore, the digestive and assimilation processes in the GI tract are well adapted for naturally occurring nano-sized substances in foodstuffs. This is because food materials contain a variety of substances that naturally exist, or are metabolised in the body, at a nanoscale. Examples of these include globular proteins, polysaccharides and lipids.[19] A concern in this regard is that nano-processing of food materials may generate nanostructures that are different from the naturally existing ones.

If nano-sized food additives have a greater ability to cross the gut wall, they may give rise to a greater internal exposure than that of the conventional equivalents. This may result in changes in nutrient profile in the body, and may potentially lead to increased health consequences in the case of certain nano-additives, such as preservatives.

The use of nano-sized delivery systems, which are designed to carry nutritional or health supplements, could also lead to introduction of foreign substances into the blood. Certain metal/oxide ENPs, such as nano-silver, are

known to have strong antimicrobial activity. It is, however, not known how their intake via food and drinks might have detrimental effects on the beneficial natural microflora in the gut. These knowledge gaps pose a major hurdle in the estimation of the overall risk to a consumer of nanofood and drinks.[6]

8.3 Potential Hazard

The application of nanotechnology derived materials in (health)food products has led to concerns that ingestion of nano-sized or nano-encapsulated ingredients and additives via food and drinks may pose certain health hazards to the consumer. This is, however, intrinsically linked to the physicochemical nature of the ENPs, and the likelihood and extent of consumer exposure through the consumption of nanofoods. It is known that nanoparticles have much larger surface areas and may exhibit substantially different physicochemical and biological properties compared to their conventional forms. As mentioned before, very few studies have been carried out so far into the toxicology of orally ingested nanomaterials, and much of the published research relates to inhalation exposure to ENPs. These studies have, nevertheless, shown that free ENPs can increasingly cross cellular barriers[20] compared to larger particulates, and exposure to some ENPs may lead to harmful effects, such as the increased production of oxyradicals that could lead to inflammatory reactions and oxidative damage to the cell.[21 23]

The modern Western diet means that the gut mucosa is continuously exposed to micro- and nano-particulates. These may be grouped into natural contaminants (e.g. soil, dust), ENPs used as food additives, and those formed *de novo* from the environment or from the gut lumen (e.g. calcium phosphate).[24] Investigations have therefore focused on finding whether there is a possible link between (micro and nano) particulate materials and exacerbated symptoms in individuals with compromised gastrointestinal functionalities (such as irritable bowel disease, IBD, or Crohn's disease). The insoluble ENPs that are not degraded in the intestine are typically taken up by M-cells of Peyer's patches and passed to underlying macrophages. As macrophages are also unable to digest the particles, it is common to see pigmentation in cells at the base of human intestinal lymphoid aggregates due to particle accumulation.[25] Concomitantly, both titanium dioxide (anatase) and aluminosilicate (as kaolinite) are commonly seen in these lymphoid aggregates.[26]

A few studies have attempted to find a relationship between the presence of (micro- or nano-sized) particulate materials in food and the initiation and/or exacerbation of certain gut diseases – such as Crohn's disease or irritable bowel syndrome. Such studies have so far focussed mainly on microparticulates, and initial findings indicate that they appear not to be indicated as stimulants for Crohn's disease or IBD when presented alone. As the particles pass through the intestinal tract, they come into contact with, and adsorb luminal constituents, such as calcium ions and lipopolysaccharide. It has been shown that microparticle-calcium-lipopolysaccharide conjugate activates both peripheral blood

mononuclear cells,[25] and intestinal phagocytes, which are usually resistant to stimulation.[27] This indicates that microparticles may be adjuvant triggers for exacerbation of disease within sufferers of Crohn's disease and IBD.[28] However, little is known about whether micro- or nano-particles are linked to the initiation of the diseases.[29] Trials carried out so far to test whether reduction of microparticles in the diet can reduce the symptoms and Crohn's disease and IBD have produced contradicting results. In a double blind randomised study, Lomer *et al*.[28] demonstrated that a particle-low diet alleviated the symptoms of Crohn's disease. However, recent clinical findings have suggested that reducing microparticle intake in Crohn's sufferers has no effect on the disease.[29] It is, therefore, evident that despite initial attempts to establish the presence or absence of a link between compromised functionality of the GI tract and initiation or exacerbation of disease, there is a requirement for considerable further research. This avenue of research should, however, in time also uncover important information about the behaviour of micro- and nano-particles within the GI system.

8.3.1 Distribution: Target Organs

The diffusion rate of particulate materials through the GI mucus is dependent on a number of factors, predominantly size, charge[30] and surface coating.[31] The translocation from the GI tract has been found to be greater for ENPs than the larger particles.[32,33] ENPs in the smaller nanometer range have been found to cross the mucus layer faster than the larger ones.[34] Again, the translocation of smaller ENPs has been shown to be comparatively greater than the ENPs in the larger nanometer range.[35] Within the GI tract, the rate uptake of ENPs has been reported to be between 2 to 200 times greater in the Peyer's patches compared with the enterocytes.[33] This is despite the fact that Peyer's patches represent only around 1% of the total intestinal surface area.

Following oral administration, the translocation and distribution of certain ENPs to different organs and tissues has been reported. Oral administration of colloidal gold ENPs (58, 28, 10 and 4 nm) to mice resulted in an increasing distribution of smaller ENPs to different organs.[35] Jani *et al*.[36] administered 12.5 mg kg^{-1} of titanium dioxide (TiO_2, rutile, 500 nm), in daily oral gavage for 10 days to female Sprague Dawley rats, and assessed their translocation to target organs and tissues such as Peyer's patches, small intestine, colon, mesentery network and nodes, peritoneal tissue, liver, spleen, heart and kidney. The authors have demonstrated that TiO_2 particles were translocated to systemic organs, such as the liver and spleen. The ENPs were also detected in the lungs and peritoneal tissues, but not in the heart and kidney. Similarly, accumulation of silver (60 nm) was observed in the stomach, followed by kidney and liver, lungs, testes, brain and blood.[37] The highest amount of intraperitoneally administered 13 nm colloidal gold beads was observed in liver and spleen.[35]

Fullerene (C_{60}) appears to pass through the placental barrier as shown after intraperitoneal administration of C_{60} fullerenes, solubilised with polyvinyl

pyrrolidone (50 mg kg^{-1}; day 18 of gestation). The C_{60} fullerenes were shown to be distributed throughout the embryo, and the yolk sac was impaired 18 hours after injection.[38] Another study, however, provides contradictory evidence. Gold ENPs, injected intravenously (2 and 40 nm) or intraperitoneally (40 nm), do not seem to penetrate the placental barrier.[39]

The conventional form of silica (SiO_2) is a permitted food additive (E551), which is used as an anti-caking and anti-foaming agent, and for clearing beers and wines. It is also used in coatings in food packaging. In an *in vitro* study on human epithelial cell cultures using fluorescence-labelled silica (SiO_2) nanoparticles, Chen and Mikecz[40] have shown that particles smaller than 70 nm could enter cell nuclei. The study also found protein accumulation in the nuclei and indication for impairment of DNA replication and transcription. It is, however, not known whether the intake of nano-silica through the GI route can lead to similar harmful effects *in vivo*.

8.3.2 Toxicity

Understanding the potential harmful and/or toxic effects of ENPs is complex. This is because the ability of ENPs to penetrate cellular barriers adds a new dimension to particulate toxicology. Thus ENPs can potentially reach new targets in the body, which are normally protected against the entry of larger particulates.

Current methods of toxicity testing rely on a range of *in vitro* and *in vivo* methods that have been developed for assessing conventional chemicals and consumer products. Whilst comparisons can be drawn from these testing regimes, many ENPs have distinctly different physicochemical properties, behaviour and interactions, which make measuring their potential toxic effects more problematic. As a consequence, it is difficult, if not impossible, to predict the effects and impacts of ENPs by extrapolating the existing knowledge on risks for larger sized particles having the same chemical composition. A major stumbling block in this regard is the lack of ability to establish the physicochemical nature and the dose of a nanomaterial to which the cells are actually exposed. One of the fundamental principles of pharmacology is that chemical activity (response) is proportional to the concentration of the affecter substance at the site of action. Unlike conventional soluble chemicals, ENPs are rarely in a homogeneous form and can aggregate or diffuse according to differences in their density, size and surface chemistry, all of which can change over time during an exposure. Consequently, there is a need to develop robust dose metrics that give a better understanding of how ENPs and different biological parameters affect both *in vitro* and *in vivo* cellular dose and corresponding toxicity profiles.

Many studies have been published which demonstrate the potential cytotoxicity of ENPs using *in vitro* cell models. Despite these reports, there are still no standardised or validated methods established for nanotoxicity testing. This has led to the publication of conflicting and confusing data and is hindering the

development of ENP risk assessment strategies. Measuring the *in vitro* dose of ENPs both at the cell surface and within cells presents analytical challenges that are hard to address with a single technology platform. Nanoscale imaging methods can measure ENP localisation and agglomeration state with varying levels of resolution, but lack the ability to quantify cell dose at the macro scale. Analytical mass spectrometry methods such as ICP-MS, LC-MS and stable isotope tracing have greater sensitivity and can quantify dose but lack information on ENP characteristics. A combinatorial approach would precipitate a more complete understanding of the factors, which influence nanoparticle dosimetry and allow for improved *in vitro* dose–response assessments.

Of the most commonly used measures of ENP cytotoxicity developed for chemical and pharmaceutical testing, only one method, the neutral red uptake (NRU) assay, is actually validated for testing chemicals under the REACH directive. Most of these 'classic' cytotoxicity and stress response assays rely on colorimetric or fluorescent outputs, which may be impacted by ENPs through absorbance of assay reagent, scattering light or quenching of the fluorescent signals. The suitability of such assays needs to be evaluated and standardised methods and protocols developed. This may, however, be further complicated by the properties of the culture system. A few studies have attempted to measure the impact of these changes. Despite such shortcomings, the *in vitro* assay systems still represent the best methods for high throughput analysis of ENP cytotoxicity, and an understanding the limitations will allow development of better methods. This has been recognised in a number of international reports by the UK's Nanotechnologies Research Coordination Group, NRCG, and recently at a European Union workshop on *Research Projects on the Safety of Nanomaterials: Reviewing the Knowledge Gaps (2008)*, which recognised the specific knowledge gaps in the field of nanotoxicology and called for urgent action to be taken to develop validated test methods for nanoparticle toxicity testing; rapid, user friendly, *in vitro* toxicity screens, and guidelines for toxicity testing.

Whilst there is a growing literature on the inhalation toxicity of ENPs, only a limited number of studies have so far been published on the toxicity of ENPs ingested through the oral route. In an oral toxicity study, Zhang *et al.*[41] fed selenite or nano elemental selenium to healthy mice at 2, 4 and 6 mg kg^{-1} bw for 12 days and found that, over a short-term, a high dose of selenite caused more pronounced oxidative stress, greater liver injury and prominent retardation of growth as compared to nano-selenium. Wang *et al.*[42] administered an equal mass dose of 5 g kg^{-1} bw of nano- and micro-sized zinc to groups of healthy mice. From the results of their experiments, the authors concluded that severe renal damage could occur in the nano-zinc treated mice, although no significant change of blood biochemical levels occurred. Nano-zinc powder could also cause severe anaemia. Wang *et al.*[43] evaluated the toxicity of ultrafine TiO_2 (155 nm) and nano-TiO_2 (25 and 80 nm) using a single dose of 5 g kg^{-1} bw delivered by gavage. The authors demonstrated that TiO_2 particles induced significant lesions of liver and kidneys in female mice. TiO_2 particles were mainly retained in liver, kidneys, spleen and lung. The hepatic injury and renal

lesions were observed in the histopathological examination. Wang *et al.*[44] evaluated the toxicity of 20 and 120 nm ZnO particles, which indicates that 20 and 120 nm ZnO, similar to the normal ZnO compound, are relatively non-toxic for mice according to GHS classification criteria. Combined with the results of zinc accumulation, pathological examination and the biological indicators assays, the target organs for 20 and 120 nm ZnO acute oral administration were demonstrated as liver, heart, spleen, pancreas and bone. The biochemical and pathological investigation showed that the toxic effects between the 20 and 120 nm ZnO particles were little different. For example, the blood viscosity could be induced by low and median dose of 20 nm ZnO, but only at a high dose of the ultrafine ZnO after oral administration. The oedema and degeneration of hepatocytes, and inflammation of pancreas could be observed in most of the 20 nm ZnO-treated mice. The 120 nm ZnO-treated mice were found having a dose–effect dependent pathological damage in gastric, liver, heart and spleen, although the 20 nm ZnO-treated mice showed less liver, spleen and pancreas damage with the increase of treated dose. Therefore, more attention needs to be paid to the potential toxicity induced by low oral doses of small-sized ZnO (e.g. 20 nm).

Silver nanoparticles (60 nm) were administered orally to groups of male and female Sprague-Dawley rats repeatedly for 28 days following the OECD test guideline 407 according to three different dosing regimens: low dose (30 mg kg^{-1}), middle dose (300 mg kg^{-1}) and high dose (1000 mg kg^{-1}).[37] This resulted in some significant dose-dependent changes in the alkaline phosphatase and cholesterol values in either the male or female rats indicating that exposure to over more than 300 mg of silver nanoparticles may result in slight liver damage.

8.4 Discussion

In this chapter we have presented an overview of the state-of-the-art issues in regard to nanomaterials and food. Since there are currently a very few established methods for detection and characterisation of ENPs in the food matrix, there is little available information on translocation from the GI tract to other organs. The available information is also very sparse in relation to dose–response data following the oral exposure. The current knowledge gaps will need studies on the following lines.

1. Analytical tools for characterisation of ENP in bulk materials and food matrices. There is a need to select appropriate reference ENPs in consideration of their likely use in food, and determine physicochemical properties, interactions, behaviour and fate in food and in the GI tract. A number of analytical methods are being developed in different research projects but they will need validating and adapting for detecting ENPs in complex food matrices as part of the routine safety testing procedures.
2. Dosimetry studies, with radio-labelled ENPs are needed. These studies will contribute relevant data regarding the translocation of ENPs,

following single or repeated oral administration, in different target organs such as liver and kidneys.

3. Dose–response studies for a range of realistic doses reflecting consumer exposure delivered by single or repeated oral administration to healthy animals to demonstrate the health effects on different target organs (e.g. lung, brain, liver, heart/circulation, etc.) and the mechanisms of action. In view of the current uncertainties over the potential toxicity hazard and exposure of ENP, it is important that these studies are carried out over prolonged exposures, and are followed by histopathological investigations on multiple organs. Other important endpoints in respect to risk assessment of ENPs include mutagenicity, carcinogenicity and reproductive toxicity. *In vitro* tests can provide a preliminary indication of the inflammatory and mutagenicity potential of the tested ENP, and if necessary, these can be followed by *in vivo* studies.

In this chapter we have also given a short overall description of the issues regarding the use of ENPs in food and food additives, and the available toxicological studies in orally exposed animals. A conceptual categorisation of different ENPs has also been presented (Figure 8.1), based on the factors that are likely to contribute to the overall risk potential of different ENPs. However, there may be other yet-unknown factors that might influence the properties and effects of ENPs. The data generated to date are sparse, and indicate major data gaps for a rigorous risk assessment to be made. The ongoing and future studies, on the lines suggested above, are likely to generate relevant data that will enable adequate risk assessment of the oral exposure to nanoparticles through the consumption of food and drinks.

References

1. Royal Society and Royal Academy of Engineering, *Nanoscience Nanotechnologies: Opportunities Uncertainties*, Royal Society and Royal Academy of Engineering, London, 2004.
2. E. Hood, *Environ. Health Perspect.*, 2004, **112**, A740–A749.
3. O. Renn and M. C. Roco, *J. Nanoparticle Res.*, 2006, **8**(2), 153–191.
4. H. Chen, J. Weiss and F. Shahidi, *Food Technol.*, 2006, **603**, 30.
5. J. Weiss, P. Takhistov and D. J. McClements, *J. Food Sci.*, 2006, **719**, R107.
6. Q. Chaudhry, M. Scotter, J. Blackburn, B. Ross, A. Boxall, L. Castle, R. Aitken and R. Watkins, *Food Addit. Contam.*, 2008, **25**, 241.
7. H. Bouwmeester, S. Dekkers, M. Noordam, W. Hagens, A. Bulder, C. de Heer, S. ten Voorde, S. Wijnhoven and A. Sips, *Health Impact of Nanotechnologies in Food Production*, Report 2007.014, published by RIKILT – Institute of Food Safety, Wageningen UR and National Institute of Public Health & the Environment – Center for Substances and Integrated Risk Assessment, 2007.

8. SCENHIR, *Risk Assessment of Products of Nanotechnologies*, 2009, http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_023.pdf.
9. EFSA Opinion on '*The Potential Risks Arising from Nanoscience and Nanotechnologies on Food and Feed Safety*', adopted 10 February 2009, available at: www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902361968.htm.
10. M. Semmler, J. Seitz, F. Erbe, P. Mayer, J. Heyder, G. Oberdorster and W. G. Kreyling, *Inhal. Toxicol.*, 2004, **16**, 453–459.
11. G. Oberdorster, E. Oberdorster and J. Oberdorster, *Environ. Health Perspect.*, 2005, **113**, 823–839.
12. Helmut Kaiser Consultancy, *Study: Nanotechnology in Food and Food Processing Industry Worldwide*, 2003–2006–2010–2015, 2004, available at www.hkc22.com/Nanofood.html.
13. Cientifica Report, *Nanotechnologies in the Food Industry*, published August 2006.
14. K. Letchford and H. Burt, *Eur. J. Pharm. Biopharm.*, 2007, **65**(3), 259–269.
15. T. M. Taylor, P. M. Davidson, B. D. Bruce and J. Weiss, *Crit. Rev. Food Sci. Nutr.*, 2005, **45**(7–8), 587–605.
16. J. F. Graveland-Bikker and C. G. de Kruif, *Trends Food Sci. Technol.*, 2006, **175**, 196.
17. M. R. Mozafari (ed.), *Nanocarrier Technologies: Frontiers of Nanotherapy*, Springer Verlag, 2007, ISBN 1402050402.
18. K. Tiede, A. B. A. Boxall, S. P. Tear, J. Lewis, H. David and M. Hassellov, *Food Addit. Contam.*, 2008, **25**, 795.
19. www.ifr.ac.uk/publications/scienceinnovation/0502_nanotechnology/.
20. M. Geiser, B. Rothen-Rutishauser, N. Kapp, S. Schurch, W. Kreyling, Y. Schulz, M. Semmler, V. Im Hof, V. Heyder and P. Gehr, *Environ. Health Perspect.*, 2005, **113**, 1555.
21. N. Li, C. Sioutas, A. Cho, D. Schmitz, C. Misra, J. Sempf, M. Wang and T. Oberley, *J. Froines and A. Nel. Environ. Health Perspect.*, 2003, **111**, 455.
22. K. Donaldson, V. Stone, C. L. Tran, W. Kreyling and P. J. Borm, *Occupational Environ. Med.*, 2004, **619**, 727.
23. E. Oberdorster, *Environ. Health Perspect.*, 2004, **112**, 1058.
24. M. C. Lomer, R. S. Harvey, S. M. Evans, R. P. Thompson and J. J. Powell, *Eur. J. Gastroenterol. Hepatol.*, 2001, **13**(2), 101–106.
25. J. J. Powell, R. S. Harvey, P. Ashwood, R. Wolstencroft, M. E. Gershwin and R. P. Thompson, *J. Autoimmun.*, 2000, **14**(1), 99–105.
26. J. J. Powell, R. S. Harvey and R. P. Thompson, *Gut*, 1996, **39**(2), 340–341.
27. P. Ashwood, A. M. Evans, F. Berisha, L. Walker-Nthenda, R. P. H. Thompson and J. J. Powell, *Gut*, 1999, **44**(suppl. 1), A92.
28. M. C. Lomer, R. P. Thompson and J. J. Powell, *Proc. Nutr. Soc.*, 2002, **61**(1), 123–130.
29. M. C. Lomer, S. L. Grainger, R. Ede, A. P. Catteral, S. M. Greenfield, R. E. Cowan, F. R. Vicary, A. P. Jenkins, H. Fidler, R. S. Harvey, R. Ellis, A.

McNair, C. C. Ainley, R. P. Thompson and J. J. Powell, *Eur. J. Gastroenterol. Hepatol.*, 2005, **17**, 377–384.
30. L. Szentkuti, *J. Controlled Release*, 1997, **46**, 233.
31. S. K. Lai, D. E. O'Hanlon, S. Harrold, S. T. Man, Y.-Y. Wang, R. Cone and J. Hanes, *Proc. Natl. Acad. Sci.*, 2007, **104**, 1482–1487.
32. M. P. Desai, V. Labhasetwar, G. L. Amidon and R. J. Levy, *Pharm. Res.*, 1996, **13**, 1838.
33. A. des Rieux, V. Fievez, M. Garinot, Y.-J. Schneider and V. Preat, *J. Controlled Release*, 2006, **116**, 1.
34. P. H. M. Hoet, I. Bruske-Hohlfeld and O. V. Salata, *J. Nanobiotechnol.*, 2004, **2**, 1.
35. J. F. Hillyer and R. M. Albrecht, *J. Pharm. Sci.*, 2001, **90**, 1927.
36. P. U. Jani, D. E. McCarthy and A. T. Florence, *Int. J. Pharm.*, 1994, **105**, 157–168.
37. Y. S. Kim, J. S. Kim, H. S. Cho, D. S. Rha, J. M. Kim, J. D. Park, B. S. Choi, H. K. Chang, Y. H. Chung, H. Kwon, J. Jeong, B. S. Han and J. Yu, *Inhal. Toxicol.*, 2008, **20**, 575–583.
38. T. Tsuchiya, I. Oguri, Y. N. Yamakoshi and N. Miyata, *FEBS Lett.*, 1996, **393**, 139–145.
39. E. Sadauskas, H. Wallin, M. Stoltenberg, U. Vogel, P. Doering, A. Larsen and G. Danscher, *Part Fibre Toxicol.*, 2007, **4**, 10.
40. M. A. Chen and A. von Mikecz, *Exp. Cell Res.*, 2005, **305**, 51.
41. J. Zhang, H. Wang, X. Yan and L. Zhang, *Life Sci.*, 2005, **76**, 1099–1109.
42. B. Wang, W.-Y. Feng, T.-C. Wang, G. Jia, M. Wang, J.-W. Shi, F. Zhang, Y.-L. Zhao and Z.-F. Chai, *Toxicol. Lett.*, 2006, **161**, 115–123.
43. J. Wang, G. Zhou, C. Chen, H. Yu, T. Wang, Y. Ma, G. Jia, Y. Gao, B. Li, J. Sun, Y. Li, F. Jiao, Y. Zhao and Z. Chai, *Toxicol. Lett.*, 2007, **168**, 176–185.
44. B. Wang, W. Feng, M. Wang, T. Wang, Y. Gu, M. Zhu, H. Ouyang, J. Shi, F. Zhang, Y. Zhao, Z. Chai, H. Wang and J. Wang, *J. Nanopart. Res.*, 2008, **10**, 263–276.

CHAPTER 9

Potential Risks of Nanofood to Consumers

HANS BOUWMEESTER AND HANS J. P. MARVIN

RIKILT – Institute of Food Safety, Wageningen UR, P.O. Box 230, 6700 AE, Wageningen, The Netherlands

9.1 Introduction

Risk assessment is the evaluation of the chance of the occurrence of harmful effects on human, animal or environmental health. The traditional risk assessment methodology comprises four stages: hazard identification, hazard characterisation, exposure assessment and risk characterisation. Health risk is defined as the combination of the likelihood of occurrence of harm to health and the severity of that harm.

This general and well-established paradigm can also be used for the risk assessment of nanotechnologies in the agri-food sector.[1] However, it needs to be acknowledged that the current state of knowledge of, for example, the toxicology of engineered nanoparticles (ENPs) is inadequate to perform a robust risk assessment.[2] In order to identify a risk and to perform a risk assessment the following information needs to be available.

- Hazard identification: the identity of the substance under investigation. For nanotechnology products, this would mean a good characterisation of the nanostructure used or the nanomaterials as present in the product.
- Hazard characterisation: the hazard induced by the substance (nanostructure) in terms of the identification of the toxicological effect.

RSC Nanoscience & Nanotechnology No. 14
Nanotechnologies in Food
Edited by Qasim Chaudhry, Laurence Castle and Richard Watkins

Published by the Royal Society of Chemistry, www.rsc.org

Subsequently the dose response of the toxicological effect needs to be assessed in order to derive a reference point.
- Exposure assessment: the exposure of an individual or a population at risk to that nanostructure.
- Risk characterisation: combining and evaluating the collected information.

While in some cases human dose–response data are available, most risk assessments rely on animal studies. The aim of the hazard identification is to identify potential critical endpoints that are of relevance for human health. Of the available data package only those studies and/or endpoints are selected that might pose a hazard to human health. This is taken one step further by considering treatment dependency of observed effects (i.e. hazard characterisation). Traditionally this is done by conventional statistical tools, e.g. pair-wise statistical evaluations of the data (e.g. the selection of the no observed adverse effect level [NOAEL]). Alternatively dose–response modelling approaches (like the benchmark dose [BMD] approach) can be used. The deduced reference point may thus be used to derive a tolerable or acceptable daily intake (TDI or ADI) after applying uncertainty factors which take into account differences in inter-species and intra-species sensitivity, usually a factor of 10 for each, resulting in a total uncertainty factor of 100. A comparison of the determined exposure level with the ADI or the TDI results in a conclusion whether there is an acceptable risk or not. This means, given the current state of knowledge, is there a reasonable certainty or not that there is no appreciable risk to human health. The final risk characterisation includes a description of the risk and the possibility of occurrence of risk based on the most sensitive observed health effect (harm).

Risk assessment is the scientific part of risk analysis. According to the general principles that have been developed by Codex Alimentarius, risk analysis can be considered as being composed of three distinct, but interacting, entities, that is risk assessment, risk communication and risk management. The scientific risk assessment, however, needs to be strictly separated from risk management in order to warrant the highest degree of scientific quality (Figure 9.1).[3]

Risk communication is considered to entail the communication about risks, related issues and the perception of risks between the professionals involved, including scientific risk assessors and risk managers, in addition to other interested groups, such as the consumer, the industry and academia. Risk management is considered to be the process in which the risks, being the outcomes of the risk assessment, are weighed against other relevant issues, such as any health benefits of the particular product in question, with the purpose of achieving the highest level of public health and fair trade practices.[3]

9.1.1 Nanotechnologies in the Agri-food Sector

Due to new, previously unknown, properties attributed to engineered nanoparticles (ENPs), many new consumer food products containing these materials have been launched onto the market recently. The potential benefits to

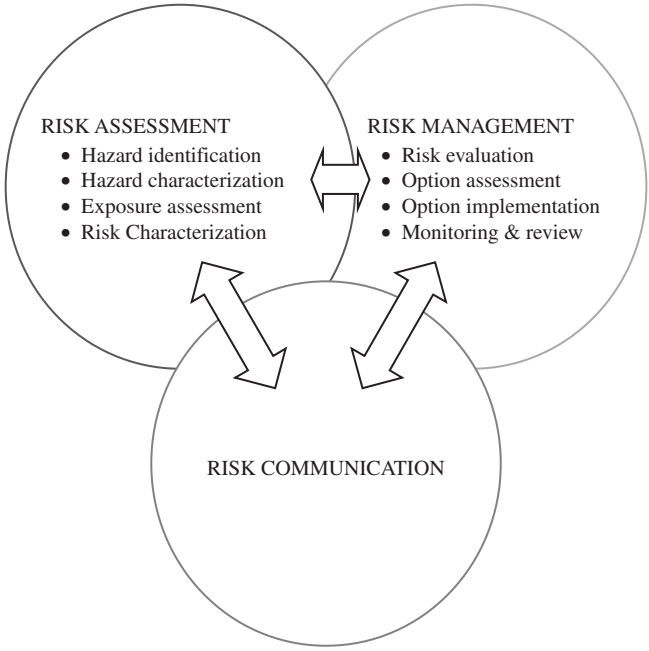

Figure 9.1 Structure of a typical risk analysis (FAO/WHO, 1997).

consumers and producers of these new products are widely recognised. Application of ENPs in electronics, medicine, textiles, defence, food, agriculture, cosmetics, and other areas is already a reality and are beginning to impact the food and associated industries.[5] In food and agricultural systems nanotechnologies may impact many aspects, such as food security, packaging materials, disease treatment, delivery systems, bioavailability, new tools for molecular and cellular biology and new materials for pathogen detection.[6,7]

Although potential beneficial effects of nanotechnologies are generally well described, the potential (eco)toxicological effects and impacts of ENPs have so far received very little systematic study. The introduction of new ENP-based products into the agri-food sector means that we need to generate a better understanding of the potential negative impacts that ENPs may have on biological systems.

In this chapter, the scientific knowledge gaps that severely hinder the risk assessment of application of ENPs in general, but specifically in food, will be addressed. Subsequently the consequences for risk assessment will be highlighted.

9.2 Knowledge Gaps for the Risk Assessment of Nanotechnologies in Food

The scientific community is global and so knowledge gaps are global too. Taking Europe as an example, the European Union's (EU) approach to

nanotechnology is intended to be 'safe, integrated and responsible'. To that end the EU has tasked its independent scientific committees and internal services to perform several activities. In 2006, The Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) was commissioned to perform a legislative review on the suitability of the existing regulation for nanotechnologies. The Committee concluded that the EU regulatory framework in principle also covered nanotechnologies.[7] While a paradigm shift was not deemed necessary, SCENIHR and others deemed necessary certain adjustments in legislation, guidelines and guidance documents concerning the testing of engineered nanoparticles (ENPs).[7] However, such adaptation can not be performed at present due to the lack of knowledge in this regard. Specifically, for the food area, these aspects have recently been considered and concluded by the European Food Safety Authority (EFSA) in their opinion on the potential risks arising from nanoscience and nanotechnologies on food and feed safety.[8] Due to the current uncertainties and gaps in the available data on hazard and exposure, the opinion recommends a case-by-case assessment of risks of the food/feed products containing engineered nanomaterials.

Discussions on data requirements and expected performance of current assays have demonstrated that it is important to focus the question on what information is additionally required compared to dossier requirements for conventional chemicals. Some research agendas or roadmaps highlight uncertainties, which are accepted in risk assessment of conventional chemicals. Another important way of focusing the discussion is to keep in mind what will really bring risk assessment to a higher level. In other words, in an area where an enormous number of research questions can be/are raised, it is essential to define those questions that represent the 'needs to know' in the context of risk assessment.

9.2.1 Physicochemical Characterisation of ENPs in Food

ENPs may encompass many forms in food. They can be made from bottom-up by assembling molecules into ENPs, or top-down by down-sizing the conventional substances. A complete and accurate characterisation of ENPs[9,10] is an essential part of both understanding the possible benefits as well as the potential toxicity of ENPs in biological systems.[11] Whereas the characterisation of chemicals is usually relatively straightforward (e.g. composition, purity), characterisation of ENPs in biological matrices is more complex.[12]

It might however not always be possible to fully characterise the ENPs. In an attempt to give some guidance on prioritisation for characterisation of ENPs, Oberdörster and co-workers[9] proposed the following three criteria:

- the context within which a material is being evaluated
- the importance of measuring a specific parameter within that context
- the feasibility of measuring the parameter within a specific context.

Specifically for food applications, it is crucial that the characterisation is performed in the matrix containing the ENPs as administered to the test

systems (or as consumed by the consumer). It is clear that the functionalities of the ENPs (e.g. particle size, size distribution, potential agglomeration and surface charge), can change in different biological matrices[10] depending on compounds that are present in the matrix and thermodynamic conditions.[12] In addition, certain interactions of ENPs with the matrix can change as a result of dilution.[9] In practice this means that the appearance of an ENP can be expected to change following (sample) processing (e.g. freeze/thaw cycles, heating, dilution).

The metrics to be determined and with relevance to risk assessment have been discussed extensively within the Organisation for Economic Co-operation and Development (OECD) and the International Standards Organization (ISO). The OECD and ISO initiatives have full governmental and industrial support. The need for standardisation of ENPs has been widely acknowledged. At present there is a vast array of analytical techniques to characterise ENPs,[9 10,13] but these methods are generally limited in that only one parameter can be assessed. Methods for *in situ* characterisation of ENPs are currently limited or lacking,[14,15] especially for the detection of nanodelivery systems.[16] Therefore research should focus on methods that are capable of *in situ* detection and characterisation of ENPs. Ideally these methods (including methods for isolation and sample preparation) should use equipment that is currently available and used in laboratories for the detection of chemical substances in food.

The development of routine analytical techniques for the characterisation of ENPs might be very difficult to achieve. In analogy with screening approaches used for complex mixtures of chemicals and products derived from genetically modified organisms in food, a completely different approach could be needed to determine the presence of ENPs, for example, by means of effect screening (e.g. *in vitro* assays). In this approach, the presence of ENPs can be 'detected' using the assay systems that focus on biomarkers of exposure or effects. The *in vitro* assays could be used in a first tier of detection of ENPs in food. However, suspected samples would have to be further characterised by means of analytical techniques.

9.2.2 Assessment of Consumer Exposure to ENPs

Exposure assessment is defined as the qualitative and/or quantitative evaluation of the likely intake of biological, chemical or physical agents via food as well as exposure from other sources (if relevant).[4]

Basically, the principle of exposure assessment of ENPs (via food) will be comparable to the exposure assessment of conventional chemicals. The actual determination of the amount and characterisation of the ENPs present in the food in its consumable form will be difficult because of the unavailability of validated methods for the detection of ENPs in food matrices. Special attention is needed for the determination of nanoscale delivery systems loaded with bioactive compounds, or bioactive compounds themselves in nanoscale formulations. Both the amount of bioactive compounds within the capsules as well

as the free form in the food matrix has to be determined, because this determines the bioavailability.

The presence of an ENP in the food matrix might result in increased bioavailability of other substances (both nutrients and contaminants) normally present in the food. For example, food containing ENPs with charged surfaces can absorb biomolecules and may act as a 'Trojan horse' for translocation through the GI tract.[17] This aspect needs to be considered when ENPs are present in food.

It will not always be feasible to measure chemicals and ENPs in the food matrix in the consumable form. If chemicals are measured at an early stage of the food chain, or at the site of production, effects of processing will also need to be considered in exposure assessment.[18]

Various sources of consumption data are currently utilised, ranging from standardised food baskets used in pre-marketing authorisations to household or individual dietary surveys used in post-marketing studies.[18] The use of ENPs as additives or in specific food products, e.g. novel foods or food supplements, might require additional data on consumption of these specific food products, as this information is generally lacking in the regular consumption databases. Although this is a general problem for exposure assessments, it is more prominent in evaluating ENPs because these particles are incorporated more frequently in food supplements.

The last step in performing the exposure assessment is the integration of food consumption and amount of chemicals or ENPs present in food. Usually one of three approaches is applied for integration of data:[18]

1. point estimated
2. simple distributions
3. probabilistic analyses.

In the end the consumer exposure is usually compared to a toxicological reference value (e.g. tolerable daily intake, acceptable daily intake or acute reference dose) or to a nutritional reference value (e.g. recommended daily intake or upper safe intake levels). These reference values are currently lacking for ENPs and need to be established.

9.2.3 Dose Metrics

It has not yet been possible to establish a single dose-describing parameter that best describes the possible toxicity of ENPs. It is likely that mass alone is not a good metric.[7] It has become clear that the size will not be the only critical factor to consider, as the total surface area may also be relevant, as well as the number of particles per particle size and possibly other characteristics. Until it is established which metrics should be used to describe the dose (e.g. particle size distribution, number of particles, particle charge, total surface),[19,20] ENPs need to be characterised extensively to allow expression of toxicological

dose–response relations using different metrics.[10,13,21] For risk assessors, it is important to have access to a clear description of the analytical methods that were used to determine the physicochemical properties of the respective ENP, and to have access to (raw) experimental data and a sound description of the statistical procedure used to analyse the data.

Discussions on definitions and dose metrics are important for several reasons. First it is important for the incorporation of nanotechnologies (e.g. the nano-size) into the regulatory frameworks that are in place to ensure the safety of food and food ingredients. Furthermore, a proper definition and dose metrics will help researchers to compare study results, as well as regulators to formulate health-based limit values. It will also enable risk assessors to compare and combine exposure and hazard information, and conclude on the likelihood of health risks. Currently pragmatic definitions are postulated stating, amongst others, that ENPs are considered as such when having size dimensions in the order of 100 nm or smaller. Future, better understanding of the mechanisms of action and dose–response relations of ENPs with biological systems will support the formulation of new and more precise definitions.

9.2.4 Toxicokinetics: Internal Exposure

Experimental data so far indicate that the characteristics of ENPs (e.g. size, surface charge, functionalised groups) are likely to influence the absorption, distribution, metabolism and excretion (ADME)[22–27] of ENPs present in food. Not much is known of the relationship between these physicochemical characteristics and the behaviour of ENPs in the body. The kinetics of ENPs following various exposure routes have recently be reviewed.[19] Although there is some data on the kinetics of ENPs following oral exposure, there is also a clear need for fundamental research on the ADME of ENPs to elucidate the driving forces and mechanisms behind these processes. In this section, we will focus on what is already known on the ADME characteristics following oral exposure, with emphasis on peculiarities of special agri-food-related uses of ENPs, including nano-encapsulates.

9.2.5 Gastrointestinal Absorption

Uptake of ENPs (or particles in general) in the gastrointestinal tract depends on diffusion and accessibility through the mucus lining of the intestinal surface, initial contact with the gut epithelium, and various uptake and translocation processes. It has been shown that smaller particles are able to diffuse faster through the mucus layer than larger particles. In addition, surface charge is also a rate-determining parameter. Anionic particles are able to reach the epithelial surface, whereas cationic particles seem to be trapped in the mucus.[28] The mucus layer can thus be considered the first barrier ENPs have to pass before entering other parts of the body.

The second barrier consists of the gastrointestinal epithelium. Cells of the gastrointestinal epithelium are tightly connected to each other by means of tight junctions. There is a body of evidence that indicates that the intestinal epithelium is permeable to large proteins and polypeptides. Moreover, the permeability of the tight junctions can be modulated, for instance by specific polymers. These polymers can act as expanders for the tight junctions thereby introducing a port of entry (e.g. paracellular uptake) for many particles including toxins, bacteria and immunogens.[27] Therefore, passage of nano-encapsulates (and ENPs in general) via tight junctions cannot be excluded beforehand, and so they should be considered by risk assessors.

The other uptake route is the transcellular route. This route describes the process by which particles are taken up at the apical side of the intestinal epithelium (by endocytosis), are transported through the M-cells in the Peyer's patches and/or the enterocytes and subsequently are released at the basolateral side of the intestinal epithelium.[23 25,29 33] Specific ENP characteristics, such as particle size, surface charge, attachment of ligands or coating with surfactants, may influence the uptake of particles by the GI tract.[33,34] If the encapsulates or ENPs are protected/prevented from local degradation or metabolism due to the modification, they will enter both the blood and lymphoid circulation intact[35] and can be further distributed in the body.

9.2.6 Metabolism and Distribution

Once the nano-encapsulates or ENPs pass the gastrointestinal epithelium and end up in the blood circulation, they can interact with various blood components (i.e. plasma proteins, coagulation factors, platelets and red and white blood cells) depending on the surface chemistry of the particle.[36] This interaction may have a substantial effect on the distribution and excretion of the particle.[37] For instance, the hydrophobic surfaces of nanospheres are highly susceptible to opsonisation (adsorption of other moieties) and clearance by the reticuloendothelial system, resulting in sequestering of the particles within organs such as the liver and spleen.[38] A widespread distribution of ENPs has also been shown with, in general, a more diverse distribution of smaller particles than the larger counterparts, e.g. to brain, bone marrow, spleen and liver.[25,32,33,39,40]

Crucial for the risk assessor is the potential passage of ENPs through natural barriers like the cellular barriers, blood–brain barrier, placental barrier and the blood–milk barrier. Cellular barriers like membranes may not constitute an obstacle for some ENPs. Phagocytosis, endocytosis, diffusion, transmembrane channels, adhesive interactions or other, undefined, transmembrane processes might play an important role in the cellular uptake. The permeability of the blood–brain barrier is restricted to molecules which are either lipophilic, actively transported or are small soluble molecules (< 500 Da). This barrier, therefore, represents a defence mechanism from blood-borne particle exposure that limits the distribution of ENPs to the brain. However, evidence exists that

distribution to the brain might occur for some ENPs.[39,40] Specific coatings of ENPs or increased surface areas of nano-encapsulates are intended to create the possibility for the development of nano-sized drug delivery systems to cross the blood–brain barrier and distribute to the brain.[41,42] Literature on the effectiveness of the placental barrier in relation to ENPs is scarce, as is the information on passage through the blood–milk barrier. The potential of ENPs to cross these barriers needs special attention in the risk assessment of the ENPs because it might impact the ultimate toxicological effects of the nanomaterials.

One of the primary aims of applying nano-encapsulates is to protect the loaded compounds from gastrointestinal and liver metabolism. At this moment, little is known on the prospect and capacity of the liver to metabolise and excrete specific ENPs. However, a size-dependent excretion of ENPs has been suggested via bile.[44] While it is unlikely that inert ENPs, such as gold and silver particles, fullerenes and carbon nanotubes, can be metabolised effectively by enzymes in the body, there are some indications that functional groups are sensitive to metabolism. For risk assessment of nano-encapsulates (and their bioactive content) it is crucial to know to what the target tissues are exposed – either the (metabolised) bioactive compound or the intact nano-encapsulate. The latter might result in the exposure of the target tissue to unmetabolised bioactive compound.

9.2.7 Potential Adverse Effects of ENPs

As the application of ENPs in foods is a phenomenon of recent years, knowledge on the potential toxicity of ENPs is limited, but rapidly growing. Several studies suggest that ENPs may have a deviating toxicity profile when compared to their conventional chemical analogues.[9,43,45] The most important question for risk assessment is the sensitivity and validity of currently existing test systems. It is generally thought that the standard battery of toxicity tests will suffice, but special attention is needed for specific endpoints.[1,46] These will be discussed in the following paragraphs, with a focus on potential toxic effects following oral exposure, and if not available also from other exposure routes.

While the literature on (acute) toxicity of ENPs is rapidly growing, it should be kept in mind that results are often obtained for only one type and size of ENP. Extrapolation from one type of ENP to another, or from one size to another, on the basis of present knowledge is not yet possible. At this moment, the knowledge base is too small to allow prediction of the nature of effects that might be expected from different types of ENPs.

9.2.7.1 In Vitro Toxicity

While results of *in vivo* studies might provide relevant information for the hazard identification of the studied ENPs, caution has to be exercised when extrapolating results or mechanisms for the hazard characterisation and subsequent human risk assessment.[47] Cells are generally exposed under artificial

conditions, and a selected number of endpoints are studied. Special attention is required for cellular interactions in order to better understand and predict cellular toxicity and the validity of the currently used *in vitro* models (e.g. for the GI absorption). While *in vitro* studies might be useful in a tiered screening approach, it is recommended to develop validated assays and assess sub-lethal changes, for example by means of profiling studies.[46,48] The search for mechanistic explanations is only now starting. For this, *in vitro* models can be very important. Numerous *in vitro* studies using different ENPs are being published. What many ENPs seem to have in common is that they can lead to an increased production of reactive oxygen species, which can cause oxidative stress and inflammatory reactions by means of interaction with the reticuloendothelial system.[45,47,49,50]

9.2.7.2 Acute Toxicity

Acute, subacute and subchronic toxicity following oral exposure has been investigated in rodents for several different ENPs (e.g. that of copper, selenium, zinc and zinc oxide and titanium dioxide). The results of the available oral toxicity studies indicate that, depending on the particle size, coating and chemical composition of the ENPs, acute toxicity at high doses may occur, but it is not always the nano form that is the most toxic form.[51 56] While results of these studies might provide some relevant information for the hazard identification of the studied ENPs, caution has to be exercised when extrapolating the results or mechanisms for the hazard characterisation to all ENPs and subsequent human risk assessment.[47]

9.2.7.3 Long-term Toxicity

Currently, there is no information available on the toxicity after chronic (long-term) or acute (short-term) low-dose oral exposure. Information from toxicity studies with other routes of exposure indicate that several systemic effects on different organ systems may occur after exposure to ENPs, including the immune, inflammatory and cardiovascular system. As yet, there is no data on these endpoints.

There is evidence from ADME studies that ENPs may pass the blood–brain barrier following systemic availability of ENPs.[39 41] It is not clear if this holds true for a subgroup only or if this a generic effect of all ENPs. Toxic effects due to the presence (or even accumulation) of ENPs in the brain have not been studied so far, but risk assessors should be aware of possible neurological effects when assessing toxicology experiments. Possibly, current guideline tests will need to be adapted to render these tests more sensitive for neurotoxic effects of ENPs.

There is some conflicting data on transfer of ENPs across the placenta.[57,58] Passage can therefore not be excluded (including excretion via breast milk – the blood–milk barrier), which could lead to embryotoxicity as a result of exposure

to ENPs.[59] Data addressing the distribution of ENPs to the reproductive cells is currently unavailable. In addition, no clear data showing the distribution of ENPs in the foetus are available.[60] This leads to the recommendation that reprotoxicity needs to be considered carefully if there is evidence for ENP passage through the placenta.

Genotoxic effects of ENPs might be driven by direct or indirect mechanisms. Intracellular ENPs do not appear to be membrane bound and might have direct access to the intracellular proteins, organelles and DNA of the cell, which might imply enhanced toxic potential.[61,62] Indirect effects might be mediated through oxidative stress responses caused by ENPs (as discussed previously). The implication of the poorly understood interactions of ENPs with cell components is that there is a need for a better understanding of the currently used genotoxicity assays and endpoints assessed.[7]

Even for conventional chemicals, little is known on the induction of food allergy and the type of exposure required to induce such responses. In the case of ENPs this becomes more prominent for two reasons. First of all it is the possible adjuvant activity of ENPs that introduces additional uncertainty.[37] And secondly, because of their charged surfaces, some ENPs can absorb biomolecules as they pass through the GI tract.[17] This so called 'Trojan horse' effect[63] may enable transportation of potentially harmful chemicals into the intestinal mucosa, resulting in changed exposure of the cellular lining of the intestine.[12] The surface properties (e.g. coatings) are important determinants for the active uptake of encapsulates, but might also be a reason for concern. For example, lectins used for coatings are highly immunogenic, can be cytotoxic, and may induce inflammatory responses and gastrointestinal irritation.[23,35,61,63]

9.2.8 Setting Health-based Guidance Values

The last step in the hazard characterisation may involve the setting of health-based guidance values such as acceptable or tolerable daily intakes. These are generally based on animal toxicity studies. Reference points (e.g. the no observed adverse effect level or benchmark dose level) for the critical effect of a substance form the starting point of the risk assessment. This is a general approach for all substances either being in a conventional form or at a nano-sized scale. It is, however, currently unknown how potential limit values derived for ENPs can be compared to those of equivalent conventional chemicals.

Furthermore, guidance values are based on toxicological studies performed with a substance (or ENP) with a given bioavailability. In this regard, ENPs are often introduced in (health)food products to enhance the bioavailability of some nutrients or bioactive compounds loaded into them. If, by some means, the bioavailability has changed (increased), this may affect the outcome of the toxicity studies and thus the calculated guidance values. Extrapolation of a health-based guidance value between formulations with different

bioavailabilities might therefore not be easy, and this might require setting of separate health-based guidance values depending on the formulation.

9.3 Consequences for Risk Analysis of ENPs

The classic notion of risk assessment requires a focus on assessing the likelihood and severity of adverse health impacts of the consumption of ENP-containing products. Current established methods for hazard and risk assessment for conventional chemicals are well adapted to achieve these goals. These methods are widely recognised, validated and accepted and implemented in harmonised regulatory frameworks. While this framework has proven effective in the past decades, it has also been a major force against innovation in risk assessment. However, such innovation may now be needed in the assessment of potential risks associated with the use of nanotechnologies in the agri-food area. Clearly, the risk assessment process needs to be embedded in a sound risk analysis procedure. Further to the technical scientific risk assessment, this includes a sound stakeholder engagement and consulting the public. It is recommended that the risk analysis is transparent with respect to the conduct and public documentation of formal assessments of the economic and social impacts. Availability of public documents on each stage of risk analysis and improved risk communication are also key recommendations towards greater transparency of the process and the accountability of all those involved in it.[64]

As indicated, ENPs have novel or distinct properties that are attributed to a combination of their small size, physiochemical properties, chemical composition and surface structure.[65] It is the added functionality of ENPs that makes them different from natural small sized particles, and from their conventional chemical counterparts. Because of this, there may also be a possibility for unexpected toxicological effects. To improve the existing risk assessment and make them suitable for ENPs, a number of aspects need addressing. These include:

- the development of validated analytical tools for the characterisation of ENPs in food matrices
- identification of the types of ENPs and (health)food products that are being developed or are already on the market to enable estimation of consumption by consumers
- establishment of agreed dose metrics to facilitate scientific studies and clear definition in the regulatory framework
- generation of knowledge in regard to kinetics and (oral) toxicity of the different ENPs
- assessment of the validity of currently used toxicological assays for ENPs. With the current state of (lack of) knowledge of toxicology of ENPs, it is unavoidable that risk assessors need as much information as possible. Over time it will be possible to evaluate the data and look for specific sets of the most relevant information. This clearly needs a closer collaboration

between the developers of ENPs and ENP-containing products, regulators and researchers. The possible need for redesigning of testing assays offers a great opportunity to explore in depth the possibilities that novel approaches like profiling technologies can offer for risk assessment of ENPs in food. These novel and innovative approaches need to be studied in parallel with the conventional techniques for validation purposes. Filling the scientific knowledge gaps also needs to be accompanied by fresh thinking in relation to risk analysis. Integration of these two can enable a successful introduction of nanotechnologies in the agri-food sector while protecting the consumer adequately.

References

1. EFSA, 2008, Draft Opinion of the Scientific Committee on the Potential Risks Arising from Nanoscience and Nanotechnologies on Food and Feed Safety. http://www.efsa.europa.eu/cs/BlobServer/DocumentSet/sc_opinion_nano_public_consultation.pdf?ssbinary=true.
2. H. Bouwmeester, S. Dekkers, M. Noordam, W. Hagens, A. Bulder, C. De Heer, S. Ten Voorde, S. Wijnhoven, H. Marvin and A. Sips, *Regulat. Toxicol. Pharmacol.*, 2009, **53**, 52–62.
3. FAO/WHO, 1997. Risk management and food safety. Report of a Joint FAO/WHO Consultation, Rome, Italy, 27–31 January 1997, FAO Food Nutrition Paper 65. 1–32. Rome. ftp://ftp.fao.org/docrep/fao/w4982e/w4982e00.pdf.
4. FAO/WHO, 1997. Food consumption and exposure assessment of chemicals. Report of a FAO/WHO Consultation, 10–14 Febraury 1997, Geneva, Document WHO/FSF/FOS/97.5, WHO, Geneva.
5. H. D. Chen, J. C. Weiss and F. Shahidi, *Food Technol.*, 2006, **60**(3), 30.
6. J. Weiss, P. Takhistov and J. McClements, *J. Food Sci.*, 2006, **71**(9), R107–R116.
7. SCENIHR, Scientific Committee on Emerging and Newly Identified Health Risk SCENIHR, Opinion on the appropriateness of the risk assessment methodology in accordance with the technical guidance documents for new and existing substances for assessing the risks of nanomaterials, European Commission Health & Consumer Protection Directorate-General, Directorate C – Public Health and Risk Assessment, C7 – Risk Assessment, 2007.
8. EFSA, Opinion on The Potential Risks Arising from Nanoscience and Nanotechnologies on Food and Feed Safety, adopted 10 February 2009, available at: www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902361968.htm.
9. G. Oberdorster, A. Maynard, K. Donaldson, V. Castranova, J. Fitzpatrick, K. Ausman, J. Carter, B. Karn, W. Kreyling and D. Lai, *et al., Part Fibre Toxicol.*, 2005, **2**, 8.
10. K. W. Powers, S. C. Brown, V. B. Krishna, S. C. Wasdo, B. M. Moudgil and S. M. Roberts, *Toxicol. Sci.*, 2006, **90**(2), 296–303.

11. The Royal Society and the Royal Academy of Engineering, *Nanoscience and Nanotechnologies: Opportunities and Uncertainties*, London, 2004.
12. K. W. Powers, M. Palazuelos, B. M. Moudgil and S. M. Roberts, *Nanotoxicology*, 2007, **1**(1), 42–51.
13. P. J. A. Borm and W. Kreyling, *J. Nanosci. Nanotechnol.*, 2004, **4**(5), 521–531.
14. K. Thomas and P. Sayre, *Toxicol. Sci.*, 2005, **87**(2), 316–321.
15. K. Tiede, A. B. A. Boxall, S. P. Tear, J. Lewis, H. David and M. Hassellov, *Food Addit. Contam.*, 2008, **25**, 795–821.
16. D. M. Luykx, R. J. Peters, S. M. van Ruth and H. Bouwmeester, *J. Agric. Food Chem.*, 2008, **56**(18), 8231–8247.
17. M. Govers, D. Termont, G. A. Vanaken and R. Vandermeer, *J. Lipid Res.*, 1994, **35**(5), 741–748.
18. R. Kroes, D. Muller, J. Lambe, M. R. H. Lowik, J. van Klaveren, J. Kleiner, R. Massey, S. Mayer, I. Urieta and P. Verger, *et al., Food Chem. Toxicol.*, 2002, **40**(2–3), 327–385.
19. W. I. Hagens, A. G. Oomen, W. H. de Jong, F. R. Cassee and A. J. Sips, *Regul. Toxicol. Pharmacol.*, 2007, **49**(3), 217–229.
20. G. Oberdorster, E. Oberdorster and J. Oberdorster, *Environ. Health Perspect.*, 2007, **115**(6), A290.
21. G. Oberdorster, E. Oberdorster and J. Oberdorster, *Environ. Health Perspect.*, 2005, **113**(7), 823–839.
22. B. Ballou, B. C. Lagerholm, L. A. Ernst, M. P. Bruchez and A. S. Waggoner, *Bioconjug. Chem.*, 2004, **15**(1), 79–86.
23. A. des Rieux, V. Fievez, M. Garinot, Y. J. Schneider and V. Preat, *J. Controlled Release*, 2006, **116**(1), 1–27.
24. A. T. Florence, *Drug Discovery Today: Technol.*, 2005, **2**(1), 75–81.
25. P. Jani, G. W. Halbert, J. Langridge and A. T. Florence, *J. Pharm. Pharmacol.*, 1990, **42**(12), 821–826.
26. B. Roszek, W. de Jong and R. Geertsma, *Nanotechnology in Medical Applications: State-of-the-art in Materials and Devices*, RIVM report 265001001/2005, 2005.
27. R. Singh, D. Pantarotto, L. Lacerda, G. Pastorin, C. Klumpp, M. Prato, A. Bianco and K. Kostarelos, *Proc. Natl. Acad. Sci. USA*, 2006, **103**(9), 3357–3362.
28. L. Szentkuti, *J. Controlled Release*, 1997, **46**(3), 233–242.
29. N. Salamat-Miller and T. P. Johnston, *Int. J. Pharm.*, 2005, **294**(1–2), 201–216.
30. M. Aprahamian, C. Michel, W. Humbert, J. P. Devissaguet and C. Damge, *Biol. Cell*, 1987, **61**(1-2), 69–76.
31. K. E. Carr, R. A. Hazzard, S. Reid and G. M. Hodges, *Pharm. Res.*, 1996, **13**(8), 1205–1209.
32. A. M. Hillery, P. U. Jani and A. T. Florence, *J. Drug Target.*, 1994, **2**(2), 151–156.
33. P. Hoet, I. Bruske-Hohlfeld and O. Salata, *J. Nanobiotechnol.*, 2004, **2**(1), 12.

34. G. J. Russell-Jones, H. Veitch and L. Arthur, *Int. J. Pharm.*, 1999, **190**(2), 165–174.
35. F. Gabor, E. Bogner, A. Weissenboeck and M. Wirth, *Adv. Drug Deliv. Rev.*, 2004, **56**(4), 459–480.
36. A. Nemmar, P. H. M. Hoet, B. Vanquickenborne, D. Dinsdale, M. Thomeer, M. F. Hoylaerts, H. Vanbilloen, L. Mortelmans and B. Nemery, *Circulation*, 2002, **105**(4), 411–414.
37. M. Dobrovolskaia, *Nature Nanotechnol.*, 2007, **2**(8), 469–478.
38. K. Letchford and H. Burt, *Eur. J. Pharm. Biopharm.*, 2007, **65**(3), 259–269.
39. J. F. Hillyer and R. M. Albrecht, *J. Pharm. Sci.*, 2001, **90**(12), 1927–1936.
40. W. H. De Jong, W. I. Hagens, P. Krystek, M. C. Burger, A. J. Sips and R. E. Geertsma, *Biomaterials*, 2008, **29**(12), 1912–1919.
41. G. A. Silva, *Surgical Neurol.*, 2007, **67**(2), 113–116.
42. P. Borm, F. C. Klaessig, T. D. Landry, B. Moudgil, J. Pauluhn, K. Thomas, R. Trottier and S. Wood, *Toxicol. Sci.*, 2006, **90**(1), 23–32.
43. K. Donaldson, V. Stone, A. Clouter, L. Renwick and W. MacNee, *Occup. Environ. Med.* 2001, **58**(3), 211–216.
44. K. Ogawara, M. Yoshida, K. Higaki, T. Kimura, K. Shiraishi, M. Nishikawa, Y. Takakura and M. Hashida, *J. Controlled Release*, 1999, **59**(1), 15–22.
45. A. Nel, T. Xia, L. Madler and N. Li, *Science*, 2006, **311**(5761), 622–627.
46. J. M. Balbus, A. D. Maynard, V. L. Colvin, V. Castranova, G. P. Daston, R. A. Denison, K. L. Dreher, P. L. Goering, A. M. Goldberg and K. M. Kulinowski, *et al., Environ. Health Perspect.*, 2007, **115**(11), 1654–1659.
47. G. Oberdorster, V. Stone and K. Donaldson, *Nanotoxicology*, 2007, **1**(1), 2–25.
48. N. Lewinski, V. Colvin and R. Drezek, *Small*, 2008, **4**(1), 26–49.
49. K. Donaldson, S. Faus, P. J. A. Borm and V. Stone, in K. Donaldson and P. J. A. Borm eds. *Particle Toxicology,* CRC Press, Taylor and Francis Group, London, 2007, pp. 299–316.
50. K. Donaldson and A. Seaton, *J. Nanosci. Nanotechnol.*, 2007, **7**(12), 4607–4611.
51. Z. Chen, H. Meng, G. Xing, C. Chen, Y. Zhao, G. Jia, T. Wang, H. Yuan, C. Ye and F. Zhao, *et al., Toxicol. Lett.*, 2006, **163**(2), 109–120.
52. X. Jia, N. Li and J. Chen, *Life Sci.*, 2005, **76**(17), 1989–2003.
53. B. Wang, W. Y. Feng, T. C. Wang, G. Jia, M. Wang, J. W. Shi, F. Zhang, Y. L. Zhao and Z. F. Chai, *Toxicol. Lett.*, 2006, **161**(2), 115–123.
54. J. Wang, G. Zhou, C. Chen, H. Yu, T. Wang, Y. Ma, G. Jia, Y. Gao, B. Li and J. Sun, *et al., Toxicol. Lett.*, 2007, **168**(2), 176–185.
55. J. Zhang, H. Wang, X. Yan and L. Zhang, *Life Sci.*, 2005, **76**(10), 1099–1109.
56. B. Wang, W. Y. Feng, M. Wang, T. C. Wang, Y. Q. Gu, M. T. Zhu, H. Ouyang, J. W. Shi, F. Zhang and Y. L. Zhao, *et al., J. Nanoparticle Res.*, 2008, **10**(2), 263–276.
57. E. Sadauskas, H. Wallin, M. Stoltenberg, U. Vogel, P. Doering, A. Larsen and G. Danscher, *Part Fibre Toxicol.*, 2007, **4**, 10.

58. M. Semmler-Behnke, S. Fertsch, O. Schmid, A. Wenk and W. Kreyling, *Proc. Euro Nanoforum – Nanotechnology in Industrial Applications*, 2007, pp. 102–104.
59. A. Fujimoto, N. Tsukue, M. Watanabe, I. Sugawara, R. Yanagisawa, H. Takano, S. Yoshida and K. Takeda, *Environ. Toxicol.*, 2005, **20**(4), 431–440.
60. C. L. Tran, K. Danaldson, V. Stones, T. Fernandez, A. Ford, N. Christofi, J. G. Ayres, M. Steiner, J. F. Hurley and R. J. Aitken, *et al., A Scoping Study to Identify Hazard Data Needs for Addressing Risks Presented by Nanoparticles and Nanotubes,* Institute of Occupational Medicine (IOM) Research Report, Edinburgh, 2005.
61. M. Geiser, B. Rothen-Rutishauser, N. Kapp, S. Schurch, W. Kreyling, H. Schulz, M. Semmler, V. Im Hof, J. Heyder and P. Gehr, *Environ. Health Perspect.*, 2005, **113**(11), 1555–1560.
62. A. V. Kabanov, *Adv. Drug Delivery Rev.*, 2006, **58**(15), 1597–1621.
63. M. C. Lomer, R. P. Thompson and J. J. Powell, *Proc. Nutr. Soc.*, 2002, **61**(1), 123–130.
64. S. P. Hong, P. R. Leroueil, E. K. Janus, J. L. Peters, M. M. Kober, M. T. Islam, B. G. Orr, J. R. Baker and M. M. B. Holl, *Bioconjug. Chem.*, 2006, **17**(3), 728–734.
65. A. König, H. A. Kuiper, H. J. P. Marvin, P. E. Boon, L. Busk, F. C. Cnudde, S. Cope, H. V. Davies, M. Dreyer, L. J. Frewer, M. Kaiser, G. A. Kleter, I. Knudsen, G. Pascal, A. Prandini, O. Renn, M. Smith, B. W. Traill, H. van Der Voet, H. van Trijp, E. Vos and M. T. A. Wentholt, The SAFE FOODS framework for improved risk analysis of foods, *Food Control*, 2008, submitted.

CHAPTER 10

Small Ingredients in a Big Picture: Regulatory Perspectives on Nanotechnologies in Foods and Food Contact Materials

ANNA GERGELY,[a] DIANA BOWMAN[b] AND QASIM CHAUDHRY[c]

[a] Director, EHS Regulatory, Steptoe & Johnson LLP, Avenue Louise 240, 1050, Brussels, Belgium; [b] Melbourne School for Population Health, University of Melbourne 207 Bouverie Street, Cavlton, Victoria 3010, Australia; [c] The Food and Environment Research Agency, Sand Hutton, York YO41 1LZ, UK

10.1 Introduction

Modern biotechnology has to date been considered by some to be the 'latest and perhaps most fundamental innovative technology to be applied to the agri-food sector.'[1] However, the convergence of nanotechnologies with the food sector is anticipated to further transform the industry.

It is therefore not surprising that significant economic growth is anticipated given recent reports. Chaudhry *et al.*, for example, reported significant advancements in relation to the development and commercialisation of processing technologies, the development of nano-structured food ingredients and delivery systems, and the commercialisation of a range of food contact

RSC Nanoscience & Nanotechnology No. 14
Nanotechnologies in Food
Edited by Qasim Chaudhry, Laurence Castle and Richard Watkins

Published by the Royal Society of Chemistry, www.rsc.org

materials (FCMs) incorporating nanoparticles,[2] with the latter, according to the European Foods Safety Authority (EFSA), likely to make up 'the largest share of current and short-term' predicted markets.[3] Further, industry commentators such as Helmut Kaiser Consultancy have suggested that the nanofood sector will, by the year 2010, be worth in excess of $US20 billion per annum.[4] This estimate may be contrasted to the predictions of another leading consulting company, Cientifica, who have suggested that by 2012, the market will be worth approximately $US5.8 billion.[5] Despite the variation in these estimates, it would appear that nanotechnologies offer industry players an economically competitive benefit and enormous potential across the entire breadth of the agri-food sector. These purported unrivalled possibilities explain the significant hype surrounding nanofoods at present. Moraru *et al*. have, for instance, asserted that nanotechnologies will revolutionise the global food supply from 'farm to fork',[6] while Arabe has predicted that future nanotechnology applications will include smart foods utilising functional encapsulation of active nanoparticles, filters that may modify flavours or remove toxins, and smart packaging that can detect the spoiling of foods.[7] Should these applications become a commercial reality, few would disagree with Lallo, when he stated that the convergence of nanotechnologies with the food sector 'seems like a Willy Wonka fantasy'.[8]

Until recently, the use of nanotechnologies within the food sector had largely escaped the intense debate that has surrounded the commercialisation of genetically modified (GM) crops within jurisdictions such as the European Union (EU).[9 14] (That is not to say, however, that the incorporation of nanotechnologies into the food sector has occurred without some debate, with a number of commentators having previously voiced their concern over the potential human and environmental health risks posed by nanotechnology applications within the food sector, and the lack of labelling thereof.) However, the high profile release of a report by Friends of the Earth (FoE) Australia Nanotechnology Project may change this altogether.[15] This report reviewed current nanotechnology applications in the food chain, and appears to have catalysed a renewed debate over the potential benefits and risks associated with the use of engineered nanomaterials within the food chain.[16 20] The comprehensive report, which documented 104 commercially available nanotechnology applications within the global food sector, focused on the current uncertainties associated with the use of the technology in the food sector, including the lack of knowledge pertaining to exposure and interactions of nanomaterials with human physiological systems and in the environment, and the potential effects thereof.

FoE suggest that the relevant regulatory frameworks in jurisdictions such as the EU, the US and Australia were inadequate to ensure the protection of consumers against these unknown risks. The authors articulated five reasons why these existing frameworks were inadequate: toxicity risks of nanofoods and agrochemicals remain very poorly understood; nanomaterials are not assessed as new chemicals; current methods for measuring exposure are not suitable for nano; current safety testing is not suitable for nano; and many

safety assessments use confidential industry studies (ref. 15 p. 39). The report, therefore, urged these political structures to call for a 'moratorium on the further commercial release of food products, food packaging, food contact material and agrochemicals that contain manufactured nanomaterials until nanotechnology-specific safety laws are established.[21] Not surprisingly, mandatory labelling laws were also advocated.

FoE's call for a moratorium on the future commercial release of nanotechnology applications in the food sector follows the footsteps of similar calls by organisations such as the ETC Group,[9] the International Union of Food, Farm and Hotel Workers[13] and the Soil Association,[12] each of whom have expressed their concern over the potential risks posed by this new technology when integrated into the food chain. The particular concerns raised by these organisations would appear to relate to the purposeful use of new, artificial nanoscale additives or ingredients in foods, and their potential effect and consequences on the digestive system, as well as the potential migration of nanoscale materials from food packaging materials, and their effect on the gastrointestinal route.

More recently, the Swiss Retailers' Association (the Interessengemeinschaft Detailhandel Schwiez or IG DHS) launched their own code of conduct for nanotechnologies.[22] The code, which has been signed by five of Switzerland's leading retailers, arguably has the potential to have a significant impact on the sale of nanotechnology-enabled consumer goods in Switzerland, including those within the food sector. The code commits its subscribers to require manufactures and suppliers of consumer goods to submit comprehensive data on products containing nanotechnologies, as well as to engage openly with consumers about products incorporating nanotechnologies.[22] The introduction of this self-regulatory initiative would appear in part to be a response to concerns over the perceived inadequacies of current regulatory regimes for managing risks,[22] and in part represent a mechanism designed to engender consumer trust for the technology in order to avoid a similar public backlash to that which occurred in Europe with respect to GM foods.[23,24] At this time it is too early to evaluate the effectiveness of the code in meeting either of these objectives.

Against this backdrop of civil society and industry driven activities, there have been a number of government-initiated and independent scoping studies that have aimed to determine the extent to which nanotechnology products and applications fall within the existing regulatory frameworks, and the adequacy of these frameworks for managing potential risks. Common to each of these reviews has been the conclusion that the majority of current and near-term nanotechnology products and applications will fall within the scope of conventional regulatory regimes. However, these frameworks do suffer from a range of 'nano-specific' regulatory gaps.

In relation to the adequacy of the US Environmental Protection Agency (EPA), Davies argued that the conventional regulatory framework, 'provide[ed] a very weak basis for identifying and protecting the public from potential risk, especially as nanotechnologies become more complex in structure and

function and the applications become more diverse'. Looking specifically at the food sector, both the UK Food Standards Agency (FSA) and Chaudhry *et al.* identified a number of areas of uncertainty and potential gaps in the relevant EU and UK frameworks. In reaching their conclusion that their 'review had not identified any major gaps in regulations', the FSA conceded that 'there is uncertainty in some areas whether applications of nanotechnologies would be picked up consistently'.[25] Taylor similarly identified a number of uncertainties and potential weaknesses in relation to the Food and Drug Administration's (FDA) ability effectively to regulate certain applications incorporating nanotechnologies within the food sector. Based on his review, Taylor concluded that 'gaps in FDA's legal authority with respect to nanotechnology products include (1) the lack of pre-market oversight tools for cosmetics, (2) FDA's inability to acquire information about nanotechnology products early enough in their development to prepare properly for their regulation and (3) inadequate authority for postmarket adverse event reporting' (ref 48, p. 7). While the FDA acknowledged in their own in-house review that 'nanoscale materials present regulatory challenges similar to those posed by products using other emerging technologies',[26] it had confidence in its ability to manage effectively the uncertainties posed by nanotechnologies under its current regime.

This chapter builds on these earlier reviews by assessing the adequacy of the EU's current regulatory frameworks for managing the potential risks posed by food and food packaging applications incorporating nanotechnologies. Such a review is timely given the dynamic nature of the European regulatory environment, including the proposal of the European Commission for a new Regulation on novel foods (COM(2007)0872), which will repeal Regulation (EC) No. 258/97. The proposal expressly includes in the Preamble 'foods modified by new production processes, such as nanotechnology and nanoscience, which may have an impact on food' as novel foods. Furthermore, the EU legislators have recently agreed on the introduction of new authorisation procedures for food additives, food enzymes and food flavourings, Regulation (EC) No. 1331/2008 which has similarly an impact on the adequacy of the regulatory regime. The relevant regulatory frameworks within the USA and Australia are also examined. Inadequacies and gaps within these instruments are considered in light of the current state of the art in terms of both science and law. In drawing conclusions, the chapter does not make assumptions regarding potential risks posed by nanomaterials at this time, nor any longer term and unimagined risks that may be posed by nanomaterials but may not be covered by the regulatory frameworks at this time. Such considerations are not only beyond the scope of this chapter, but also the health and safety risks of any such hypothetical unforeseen risks would appear to fall beyond current scientific understanding. Finally, in acknowledging the delicate balance between promoting innovation whilst taking reasonable precautions in order to protect the public, the chapter articulates a range of mechanisms that could be adopted to assist governments in achieving this fine balance.

Due to the dynamic nature of the regulatory framework in all of the jurisdictions considered within this chapter, it is important to note that the

regulatory review undertaken for the chapter was correct at the time of writing, September 2009.

10.2 Regulatory Review: European Union

10.2.1 General food Safety and Consumer Health Protection

EC Food Law Regulation 178/2002 sets down the general principles and requirements of food law within the EU. The Regulation provides for the establishment of the European Food Safety Authority (EFSA) (Article 22), sets down the procedures in matters of food safety, and hence provides the basis for the assurance of high level of protection of human health and consumers' interest with respect to food.

Pursuant to Article 14(1), food cannot be placed on the market 'if it is unsafe', moreover it is ineligible for marketing if it contains substances harmful to health. Regulation 178/2002 further stipulates that the ultimate responsibility for ensuring that the final food product is safe rests with the seller who offers the food or packaged food for sale, or who offers unfilled materials or articles for sale to consumers for home use (Article 17). Due to the inclusive nature of Regulation 178/2002, the general safety articles embodied therein will, by implication, encompass foods containing nanomaterials and/or manufactured using nanotechnologies. Consequently, the notion of traceability of nanomaterials as food ingredients or additives is covered under the existing requirements of Regulation 178/2002.

10.2.2 Regulatory Aspects Relating to Nanoscale Food Ingredients

Besides the Food Law Regulation and its general safety criteria, EU legislation of particular relevance to nanoscale food ingredients is Regulation (EC) 258/97 concerning Novel Foods and Novel Food Ingredients. This establishes a mandatory pre-market approval system for all novel foods. According to the European Commission (EC), 'Regulation 258/97 allows assessing possible risks associated with the use of nanomaterials and nanotechnologies (novel food ingredients) and nanotechnologies (novel technology with impact on food) in relation with food and food ingredients'.[27] This Regulation is currently under review, and is going to be replaced with a new Regulation which 'aims to streamline the authorisation procedure, develop a more adjusted safety assessment system for traditional food from third countries ... and clarify the definition of novel food, including new technologies with an impact on food'.[28] As the recast of the Regulation is still not adopted, this chapter will focus on the current regulatory framework; however, reference will still be made to the proposed Regulation, due to the express inclusion of 'nanotechnology and nanosciences' within its text.

Pursuant to Article 1 of Regulation 258/97, a 'novel' food is defined as a food or food ingredient not having a significant history of human consumption within the Community prior to May 1997 and which falls within one of several defined categories. The categories that may have relevance to nanotechnologies include:

- 'foods and food ingredients with a new or intentionally modified primary molecular structure' (Article 1(c) Regulation 258/97)
- 'foods and food ingredients to which has been applied a production process not currently used, where that process gives rise to significant changes in the composition or structure of the foods or food ingredients which affect their nutritional value, metabolism or level of undesirable substances' (Article 1(f) Regulation 258/97).

Considering the current and projected applications of nanotechnologies in food, it is unlikely that most nano-structured food products (at least in the foreseeable future) would fall under the first category, that is, they would not necessarily have a different molecular structure compared to normal processed food. The foods would be 'enhanced' or 'modified' rather than being different at the molecular level, though this possibility cannot be ruled out. There is, however, a stronger and more immediate likelihood that they would fall under the second category, providing that the attached caveat is fulfilled. The onus for recognising that a food substance falls under the latter category and alerting the competent food assessment body lies with the person responsible for placing the product on the market. However, in the case of nano-structured foods, Regulation 258/97 would only appear to be applicable if a substance was regarded both as 'novel' and its nutritional value, metabolism *or* level of undesirable substances was substantially altered compared to its macro-scale counterpart.

If the company responsible for placing a nanofood product on the market did not recognise it to be novel (because, for example, the ingredients already have a history of use at the macro-scale) and/or did not consider the properties of the nanofood to be substantially different from its macro-scale counterpart (due, for example, to a lack of information to the contrary or the lack of a precise definition of the term 'substantially altered'), then it is possible that a safety evaluation under Regulation 258/97 would not be carried out. This means that the caveat attached to the definition of this category of novel foods under the current Regulation leads to uncertainty over whether a nano-structured food product falls into this category or requires testing to show that their nutritional value, metabolism or level of undesirable substances have not been affected. It is also unclear whether this regulatory framework would apply to food ingredients that have a significant history of use but may already be marketed (by chance, not by design) in forms that contain particle sizes of 100 nm or less.

The proposed recast of the Regulation provides the EC with a unique opportunity to address the current ambiguities of Regulation 258/97, especially in relation to nanofood products. Under the final draft proposal, published in

January 2008, the definition of a 'novel food' is altered so as specifically to include 'food to which is applied a new production process, *not used before 15 May 1997*, where that production process gives rise to signification changes in the composition or structure of the food which affects its nutrition value, metabolism or level of undesirable substances' [emphasis added].[29] A new production process is defined so as expressly to include 'foods modified by new production processes, such as nanotechnology and nanoscience'.[30] What is meant by 'nanotechnology and nanoscience' is not defined in the current proposal. However, as with Regulation 258/97 the proposed Regulation applies only if the food's nutritional value, metabolism profile or the level of undesirable substances was substantially altered relative to its macro-scale counterpart. Only when both criteria are fulfilled would the food be considered to be 'novel food' for the purposes of a pre-market safety evaluation and require listing on the proposed Community List of novel foods.[31]

The proposed framework did not overcome the current uncertainties associated with Regulation 258/97 in relation to whether or not the regulatory instrument applies to food ingredients that have been used before 15 May 1997 which have already been marketed (by chance, not by design) in forms that contain particle sizes of 100 nm or less. The opportunity to clarify this situation within the text of the new Regulation existed in relation to nano-structured foods. In the view of FoE, the recast 'could have provided an opportunity to change the legislation to cover nanofoods properly'.[32]

10.2.3 Regulatory Aspects Relating to Nanoscale Food Additives

The use of food additives is currently controlled by Framework Directive 89/107 (the 'Food Additives Framework Directive') and the subordinate legislation. Nanofood additives are assessed either as novel additives or, where a macro-equivalent is already approved, through potential amendments of the appropriate specifications, including purity criteria, under the Directive 2008/84/EC.

From 2010 on the Food Additives Framework Directive will be replaced by a common authorisation system, with the European Community having adopted a set of Regulations, which will provide for a common basis of controls on food additives (Regulation (EC) No. 1333/2008), food enzymes (Regulation (EC) No. 1332/2008), and food flavourings (Regulation (EC) No. 1334/2008). Moreover, the adoption of the common authorisation procedure will bring together all of the existing food additive regulations, and introduce comitology for the approval of the three categories of substances. Moreover, in line with the decision to separate risk assessment and risk management, under the new system, all applications for the approval of each category of substance will be directed to EFSA, who will carry out the safety evaluations and risk assessment. Pursuant to the new Regulations, a positive list ('Community list') will be established for each substance category. As noted by the European Commission, 'the inclusion of a substance on one of these lists means that its use is authorised in general for all operators in the Community'.[33]

Note that a 'food additive' is defined (in Article 3(2) Regulation (EC) No. 1333/2008) so as to mean 'any substance not normally consumed as a food in itself and not normally used as a characteristic ingredient of food, whether or not it has nutritive value, the intentional addition of which to food for a technological purpose in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food results, or may be reasonably expected to result, in it or its by-products becoming directly or indirectly a component of such foods; . . . '

In addition to the safety of the substance, the other general criteria (technological need, consumer aspects) will have to be examined before a food additive may be included in the positive list. Under the new Regulation, this would be done by the Standing Committee on the Food Chain and Animal Health (SCFCAH). For an authorised food additive to be included on the proposed positive list, a specification must be laid down that contains the criteria on purity and defines the origin of the food additive or enzyme, and the verification of such criteria (for food additives, see Preamble 8 and Article 14 Regulation (EC) No. 1333/2008; for food enzymes, see Preamble 8 and Article 7 Regulation (EC) No. 1332/2008).

The most relevant aspect in relation to the use of nanoscale food additives in the new Regulation is arguably the re-evaluation of safety assessment, which will ensure that food additives, once permitted, are kept under continuous observation and re-evaluation. Therefore, under the new Regulation, producers or users of food additives which are 'significantly different from those included in the risk assessment of the Authority or different from those covered by the specifications laid down' will be obliged to inform the Commission of any new information that may affect the safety assessment of a food additive. As stated in the proposal, a 'significant difference' could mean *inter alia* a change in the manufacturing process or in specifications, changing conditions of use, any new scientific information, or 'a change in particle size' (see Preamble 12 of the Regulation (EC) No. 1332/2008 in relation to food enzymes). Based on the new definition, the use of nanotechnology will constitute a 'significant difference' for the purpose of re-evaluation by the EFSA, and is an important inclusion in the new Regulation. Moreover, under the new Regulation, the EFSA will also be invested with the power to re-evaluate a food additive on the basis of 'new scientific information' (Preamble 14 of Regulation (EC) No 1333/2008). While it is unclear at this time whether or not 'new scientific information' would be interpreted so as to include development in nanotechnologies, it is argued that the express inclusion of 'change in particle size' in the new Regulation may be relevant for triggering re-evaluation by the EFSA.[27]

A potential limitation of the proposed regulatory regime in relation to nanoparticles appears to be the current lack of information to describe adequately the food additive; that is, to ensure that all relevant aspects that correspond to the additive have been assessed for safety. While it is provided that the previous food additive specifications would be maintained until the corresponding additives are entered into the Annexes of any new Regulation, there are as yet no criteria within the specifications that cover the use of nanoparticles

per se. For example, in the case of a coating intended to provide moisture or oxygen barrier to confectionery products (Mars Inc.'s US patent US5741505), the purity specification for silicon dioxide (E551) describes only the process by which SiO_2 may be produced for food additive use (in other words, no definitions for source materials are prescribed). However, the source compounds for SiO_2 used in the production of the nanoscale SiO_2 coatings includes organosilicates, silanes, chlorosilanes and tetraethylorthosilane. In addition, the current EU purity specification for SiO_2 (E171) does not prescribe criteria related to particle size, which clearly is a principal issue in terms of the use of nanotechnologies. This additive was last evaluated in 1977.

Food processing aids, specifically, are not included within the scope of the new Regulation. This may have implications on the use of certain nanotechnologies. For example, carrier systems used to protect additives during processing only appear under the auspices of novel foods. It is clear that food additives must at all times comply with the approved specifications. The definitions laid down in Article 3(2) of the new Regulation list certain substances that are not to be considered as food additives. Among these are substances being used as, for example, nanomicelle-based carriers, which have the potential to lead to a blurring of the distinction. This occurs, for example, in certain types of dextrin and modified starches, gelatine and products containing pectin.

See Article 2 of Regulation (EC) No. 1333/2008: pursuant to Article 3(b), 'a 'processing aid' shall mean any substance which: (i) is not consumed as a food by itself; (ii) is intentionally used in the processing of raw materials, foods or their ingredients, to fulfil a certain technological purpose during treatment or processing; and (iii) may result in the unintentional but technically unavoidable presence in the final product of residues of the substance or its derivatives provided they do not present any health risk and do not have any technological effect on the final product; . . . '

10.2.4 Regulatory Aspects Relating to Food Contact Materials (FCMs)

Good manufacturing practice (GMP) requirements are set forth in Commission Regulation (EC) No 2023/2006 of 22 December 2006 on good manufacturing practice for materials and articles intended to come into contact with food, which became applicable from 1 August 2008. The GMP Regulation applies to all sectors and all stages of manufacture of food contact materials, except starting substances. The elements of GMP are defined as the establishment, implementation and adherence to appropriate quality assurance, quality control and documentation systems.

Food contact materials (FCMs) are subject to the requirements of Regulation (EC) No 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food (the Framework Regulation). Article 3 of the Framework Regulation requires

that all FCMs be manufactured in accordance with good manufacturing practice so that, under normal or foreseeable conditions of use, they do not transfer their constituents to food in quantities that could endanger human health, bring about an unacceptable change in the composition of food, or deteriorate the organoleptic characteristics of food. The Regulation also allows for the adoption of specific measures for a group of materials and articles identified in Annex I to the Regulation. Plastics are among the groups of materials for which specific measures have been adopted under the Framework Regulation.

Plastics are subject to the Commission Directive 2002/72/EC of 6 August 2002 relating to plastic materials and articles intended to come into contact with foodstuffs, known as the Plastics Directive. The Plastics Directive applies to materials comprised exclusively of plastics, and subjects plastic food-contact articles to the overall migration limit (OML) set forth in Article 2 of the Directive, which prohibits an overall, cumulative, migration into contacted food of all constituents of the finished plastic article in quantities exceeding $10\,mg\,dm^{-2}$ or $60\,mg\,kg^{-1}$.

The Plastics Directive also sets forth a positive list of authorised monomers (Article 3) and additives (Article 4) that may be used in the manufacture of plastics. The list of permitted monomers and other starting substances of the Plastics Directive is a strict positive list. That is, only substances listed in Annex II of the Directive are permitted for use as starting substances in the manufacture of plastics. However, the list of permitted additives in Annex III of the Directive is currently considered to be incomplete. This means that unlisted additives may be used today in compliance with applicable EU legislation, provided that they are safe for their intended use and that these additives and the finished product in which they are used comply with the applicable national legislation of the Member States in which they are marketed, subject to the principle of mutual recognition.

The principle of mutual recognition allows for the legal importation and sale in a Member State of products that are legally marketed in another Member State even if the products do not comply with the specific regulatory requirements of the country of import, unless authorities of the country of import can demonstrate that the products raise legitimate health or safety concerns. A Regulation confirming the applicability of the principle of mutual recognition to all industrially manufactured or agricultural products, and establishing minimum procedural guarantees for companies marketing their products on the basis of mutual recognition, was adopted by the Council of the European Union on 23 June 2008. The Regulation has entered into force 9 months after its publication in the Official Journal of the European Union.

However, the fifth amendment to the Plastics Directive, Directive 2008/39/EC (Commission Directive 2008/39/EC of 6 March 2008 amending Directive 2002/72/EC relating to plastic materials and articles intended to come into contact with food, OJ L63, 7.3.2008, p. 6.), establishes the date when the additives list becomes a positive list as 1 January 2010. As of that date, only additives listed as permitted components in the Directive, and additives listed in

the provisional list of components that were petitioned for inclusion into the Directive before 31 December 2006, can be used.[34]

In addition to the condition of the additives list, various exemptions from the positive listing requirement currently exist for both monomers (Article 3.5) and additives (Article 4.3). These exemptions are based on the applications in which they are used. Of course, in all cases the substances present in the food contact plastics must still permit the finished plastic to comply with the general requirements of the Framework Regulation (safety, no unacceptable change in composition or deterioration of the quality of the food), even if they are not required to be listed in the Directive. Because these exempted uses fall outside of the scope of harmonised EU law, they must also comply with applicable national legislation of the Member States in which they are marketed, subject to the principle of mutual recognition.

The most straightforward way of demonstrating that a substance used in the manufacture of FCMs complies with the general safety requirements of Article 3 of the Framework Regulation is to establish that it is covered by an applicable listing in the Plastics Directive and that it is used in accordance with the potential restrictions that may be established for that substance under the Directive. Indeed, it is the very purpose of the Directive, as a specific measure under the Framework Regulation, to establish requirements implementing the general safety requirements of the Regulation when applied specifically to food contact plastics. The listing of a substance as a permitted component of plastics under the Directive presupposes a review and determination by the EFSA or its predecessor the EU Scientific Committee on Food (SCF) that the substance could be considered safe in the applications of interest provided potentially applicable restrictions are met.

For the same reason, a reference to a listing in the Plastics Directive is another effective way of demonstrating that a substance is 'safe' and therefore, in compliance with Article 3 of the Framework Regulation. This should occur even if the substance is intended for use in an application that is not subject to the Plastics Directive (for example, rubber, paper and board or silicones) or is intended for a use that is not covered in the positive list of authorised monomers and additives (for example, adhesives, coatings, or solvents). This is especially true in several Member States where the lists of authorised substances in the Directive, as implemented into their national legislation, may be applied to materials that are beyond the scope of the Directive but subject to national positive list requirements. Based on the foregoing, a Plastics Directive listing, even when not required under EU law, may be used to establish safety and compliance with national requirements.

Substances that are not listed in the Plastics Directive can be demonstrated to be safe by reference to a favourable listing in a national positive list of an EU Member State. Alternatively, other national positive listings can be considered that may not be directly applicable to the intended conditions of use of the substance, but that nevertheless may have some relevance to the safety of the material by, for example, clearing its use in similar applications or in applications that would result in similar levels of exposure.

Alternatively, a conclusion of safety can be based upon a favourable listing by a competent authority of another (non-EU) country, such as the US FDA, or recommendations of intra-governmental organisations such as the Council of Europe, or upon a self-assessment providing a conclusion based upon well-accepted scientific principles.

Hence, under the existing regulatory scheme FCMs have to be safe for the intended application in all conditions. To establish safety is the responsibility of the manufacturer, as clearly required by the GMP Regulation.

10.2.5 Regulatory Status of FCMs Produced by Nanotechnologies

Nanomaterials when used in food contact applications do fall under these same requirements; that is, independent of an existing potential clearance for the bulk analogue, the intended use of the nanoform as a FCM should be demonstrated to be safe. As described above, the demonstration of safety is the duty of the manufacturer, hence it is the manufacturer's responsibility to provide packaging materials using nanotechnology, which can be proven to be safe in the intended applications. According to Article 16 of the Framework Regulation, there is a requirement on the declaration of compliance, requiring that materials and articles for which specific measures have already been established (such as plastics) should be accompanied by a written declaration stating that they comply with the rules applicable to them. The appropriate documentation proving this compliance shall be available to the competent authorities on demand. For applications where specific measures do not yet exist, Member States may retain or adopt national provisions for demanding declarations of compliance for materials and articles. This means that explicit provisions already exist now in the Framework Regulation, which demand that materials and articles used in food contact applications are to be demonstrated to be safe and suitable for the intended use on a case-by-case basis. If the safety of a material is not fully known, its use can only be demonstrated to be safe if there is information to document the lack of migration and/or exposure to the substance at any toxicologically significant level. Consequently, the manufacturers and users of nanomaterials or any products of nanotechnologies in food contact applications have to either:

1. have sufficient toxicological information on the nanomaterial to demonstrate safety to human health, or
2. be able to demonstrate the lack of migration to prove that the intended use will not result in the transfer of these constituents to food in quantities that could endanger human health, bring about an unacceptable change in the composition of food, or deteriorate the organoleptic characteristics of food.

However, as described above, for certain types of FCMs a positive evaluation of the material in the intended use is needed by the EU Authority, EFSA,

resulting in the authorisation of that substance in the supported applications. The present Annexes to the Plastics Directive contain a few hundreds of such 'cleared' substances. Cleared substances include monomers and additives which the Commission has authorised for use in specific food contact plastics applications following the risk assessment performed by EFSA. Importantly, the preamble to the Directive declares that,

. . . the establishment of a list of approved substances accompanied by a limit on overall migration and, where necessary, by other specific restrictions will be sufficient to achieve the objective laid down in Article 2 of Directive 89/109/EEC (the present Article 3 requirements of the Framework Regulation).

On that basis for substances already listed in the Annexes of the Directive it seems to be sufficient to only adhere to the potential restrictions and as such compliance with the general safety requirements of the Framework Regulation is guaranteed.

Some of these authorised substances, however, may exist in nano forms and – as the size was not a characteristic in the past that triggered any specific regulatory attention – almost no restrictions exist today that relate to the particle size of the authorised materials. On that basis it can be expected that companies – relying lawfully on the authorisation of a substance as a food contact additive (based on a petition covering its bulk form) – would claim to use the nano form in full compliance with the existing regulatory requirements. Indeed, the above cited statement from the preamble seems to pre-empt the need to demonstrate safety by any other ways when a listed substance is used in permitted applications observing the restrictions of that listing.

However, communications from Commission officials including the draft recast of the Directive suggest that using a material in its nano form on the basis of its listing in bulk form is not acceptable and the nano version would require a new separate safety assessment (on the basis that it has been produced by a different process than that used for conventional material). This position, if consequently applied, would result in the very difficult task of establishing whether a new physical form of a substance should be considered 'nano' and requiring further petitioning. This will be particularly difficult given there is no common agreement of how 'nano' can be defined and what are the very characteristics that make the behaviour of a nanomaterial so different from its bulk counterpart.

Nonetheless, there is one general provision in the Framework Regulation, which reassures that relying on an existing authorisation of a bulk substance would not facilitate the irresponsible use of its nano form. Indeed, Article 11(5) of the Framework Regulation provides that:

The applicant or any business operator using the authorized substance or materials or articles containing the authorised substance shall immediately inform the Commission of any new scientific or technical information, which might affect the

safety assessment of the authorised substance in relation to human health. If necessary, the Authority shall then review the assessment.

Commission officials argue that using a nano form of an authorised substance is covered by this provision, possibly obliging the user/manufacturer of the nano form to inform the Commission of the application, even if potentially there is no new scientific or technical evidence questioning the safety of the nano form in comparison with its bulk equivalent. Certainly it would be necessary to receive clear guidance on that point from the Commission in order to decide on the petitioning obligations for the nano forms of existing, authorised bulk food contact substances.

As described earlier, the legislation for FCMs is only partially harmonised; the evaluations by EFSA are only mandatory for monomers and other starting substances used to manufacture polymers in food contact applications made entirely of plastics. By January 2010, the same mandatory evaluation will, however, be in place for all additives used in these applications. This means that in these applications no substance can be used – in nano or in bulk form – unless its risk has been assessed by the EFSA and its intended use authorised, including possibly substances on the positive list of permitted substances with the appropriate restrictions. Hence, this regulatory change would extend the scope of those applications where direct pre-market authorisation is required, calling for:

1. clarifying the above confusion of relying on existing authorisation for bulk substances, potentially supported by the scientific belief of the manufacturer that the use of the substance in nano form would not compromise the safety evaluation; and
2. developing proper criteria for the safety evaluation of nanomaterials allowing their petitioning and appropriate listing as authorised substances in food contact plastics applications.

These action items are in our view probably too demanding in the framework of the current authorisation procedure. Today, if following the evaluation of a detailed submission on potential migration of the petitioned FCM in the intended applications and the documented toxicology of the substance, the EFSA concludes, that the use of the material is safe, it recommends to the Commission the inclusion of the substance on the positive list of permitted substances. The Commission may follow the risk assessment of EFSA and update the appropriate Annexes of the Directive (Annex II for monomers and Annex III for additives) listing the evaluated substance with the appropriate restrictions on the use level/maximum migration limit (specific migration limit, or SML) and other relevant restrictions.

However, as explained in detail above, any future manufacturer and user of the substance can then rely on this authorisation, provided the restrictions are correctly observed. As future manufacturers have no information on the actual description of the substance and its authorised use conditions other than those

specifically listed as specifications and/or restrictions, they are even theoretically not in the position to properly judge whether or not relying on an existing listing goes beyond what the safety evaluation of the substance has actually addressed. The other side of this same token is that when EFSA concludes the risk assessment for a substance, and the Commission issues the relevant restrictions for its use, they have to foresee all other potential uses – and possibly, misuses – of the substance by future users who would only need to comply with the restrictions as worded by the regulators. This is clearly a huge burden for the authorities and can only be possibly handled by 'over-regulating' the substance and going much beyond its potential impact required by the application as petitioned.

The risk assessment for nanomaterials may require to break from this existing approach. It is not feasible scientifically to regulate different applications via general restrictions established on the basis of certain limited testing for one specific application, which is claimed to be 'representative'. This is because we may not know enough about the behaviour of nanoparticles, and therefore, we may unable to extrapolate the information gained on one specific nanomaterial to another application. Moreover, we also do not understand what are the specific characteristics of a nanoparticle that need to be kept under control. Listing a nanoparticle on the positive list on the basis of one petition describing one specific use is scientifically probably not supportable, hence the authorities may need to re-think the applicability of a general positive list based system for food contact applications involving nanotechnologies altogether.

Instead, as for other food contact applications where the positive list system has not yet been developed into a harmonised system, the safety of the application should rather be established on a case-by-case basis, using sound scientific principles based on relevant data on toxicology versus exposure and resulting in an application-specific proprietary clearance for the actual application in question.

10.2.6 European Activities relating to Nanotechnologies in Food and FCMs

Against this backdrop, in 2007 the EFSA Scientific Committee received a request from the European Commission, pursuant to Article 29 of Regulation (EC) 178/2002, for a scientific opinion on 'risks arising from nanoscience and nanotechnologies on food and feed safety and the environment' (Question No EFSA-Q-2007-124). As noted by the EFSA, 'the request also asks to identify the nature of the possible hazards associated with actual and foreseen applications in the food and feed area and to provide general guidance on data needed for the risk assessment of such technologies and applications'.[35] The EFSA opinion, published in March 2009,[36] highlighted the range of uncertainties and limitations thereof associated with risk assessment for

nanotechnologies when used in food and feed. These limitations, as outlined by EFSA, included:

1. difficulties associated with the characterisation, detection and measurement of engineered nanoparticles in food and feed
2. the lack of data on (likely) exposure from likely applications within the agri-food sector
3. the lack of data relating to potential environmental impacts associated with engineered nanoparticles in food and feed, and
4. limited data on the current usage levels of engineered nanoparticles in food and feed.[37]

Despite these seemingly significant gaps in scientific knowledge, EFSA concluded that 'the currently used risk assessment paradigm . . . is considered applicable for ENMs [engineered nanomaterials]',[38] while advocating a case-by-case approach for risk assessment of food and feed incorporating engineered nanomaterials.

While the terms of reference for the opinion did not expressly include regulatory considerations within their scope, the generic nature of the draft opinion in relation to risk assessment and the recommendations arising out of the report appear unlikely to have any significant implications for the EU regulatory framework in the near future.

In addition to the aforementioned draft scientific opinion, the EFSA has issued an opinion on a nanomaterial applied as a coating on a food contact material.[37] The material in question is a silicon dioxide coating (SiOx) formed from the monomers hexamethyldisiloxane and hexamethyldisilazane that are used to make a silicon dioxide coating *in situ* on the inner surface of PET articles. The coating is intended to provide gas barrier properties and the maximum thickness is 100 nm. The final articles are intended for all types of food, including hot fill and pasteurisation at temperatures up to 95 °C, and long-term storage at room temperature.

According to the EFSA option, the data provided with the submission included:

- physical/chemical data: identity, physical and chemical properties, hydrolysis studies, use and authorisation, analysis for migrateable oligomers or other reaction products, determination of residual content
- toxicity data: gene mutation in bacteria (two assays), *in vitro* mammalian cell gene mutation test, chromosome aberration tests (two assays), alkaline elution assay, sister chromatid exchange assay, *in vivo* mammalian bone marrow chromosome aberration test and cytotoxicity test in mouse lymphoma cells.

The evaluation by EFSA considered that hexamethyldisilazane is not stable in moist air or in aqueous media and is converted rapidly into hexamethyldisiloxane. The residual content of hexamethyldisiloxane in PET bottles treated with

both monomers to give a 43 nm barrier coating was 0.9 microgram per 6 dm^2. No other constituents with a molecular mass below 1000 g mol^{-1} were detected, using LC-MS. Overall migration was not determined, but given the very low thickness of the coating this information was deemed not required. Other (non-nano) components considered were bis(2,6-diisopropylphenyl)carbodiimide, 89120 (stearic acid, butyl ester) and 70480 (palmitic acid, butyl ester).

The EFSA opinion showed that, based on the data provided, the substance is classified as SCF_List: 3, with restrictions to 0.05 mg kg^{-1} of food (measured as hexamethyldisiloxane), and may only to be used as a surface treatment agent on PET. An added remark for the Commission stated that 'only a method of analysis for the determination of the residual content of hexamethyldisiloxane on the treated surface is provided'.[39] The document did not identify any requirement for more data or information.

An important aspect of this first EFSA opinion on a nanomaterial in a FCM is the statement 'overall migration was not determined but given the very low thickness of the coating this information is not required'.[40] This is likely to have bearing for future assessments on other nanomaterial based coatings on FCMs.

Another FCM containing nano-sized titanium nitride (TiN) has also been considered by EFSA. In this application the nanomaterial is intended for dispersal in a polymer matrix as a processing aid (for uniform heat dissipation during thermo-formation of objects). The EFSA opinion concluded that as titanium nitride as such is chemically inert and completely insoluble in all food simulants, its intended use would not give rise to exposure via food, hence it does not represent any toxicological concern. The opinion further stated that on that basis TiN nanoparticles can be used in PET bottles up to 20 mg kg^{-1} without providing any further toxicological data on the substance. However, the adoption of a favourable opinion by EFSA does not guarantee the automatic listing of the substance in the Plastics Directive; as based on Article 11, paragraph 2 of the Framework Regulation, the Commission is not legally obliged to act upon a favourable EFSA opinion authorising the use of the substance at the EU level. Instead, the article specifies that the Commission can consider 'other legitimate factors relevant to the matter under consideration', but has to inform the applicant without delay and justify its decision not to follow the recommendations of the EFSA opinion. It will therefore be interesting to see how the Commission is going to follow up the above EFSA opinions on the food contact applications of nanomaterials.

10.3 Regulatory Review: United States

10.3.1 General Food Safety and Consumer Health Protection

The US legislative instrument of most relevance to this chapter is the Federal Food, Drug and Cosmetic Act (the FDC Act), which is administered by the Food and Drug Administration (FDA). (For the purposes of this chapter, other relevant legislative instruments include the Dietary Supplement Health

and Education Act of 1994, the Food Additive Amendment Act of 1958 and the Code of Federal Regulations.) The FDA also has jurisdiction over other product categories, including drugs, devices and cosmetic products. In relation to foods, the FDA – through its Center for Food Safety and Applied Nutrition – is required by law to ensure that foods that fall within the regulatory scope of the FDC Act are safe. Pursuant to the definition of a food, as set out in s.201(f) of the FDC Act, this includes whole foods, food additives, dietary supplements, 'generally recognised as safe' (GRAS) food ingredients and FCMs. This is achieved through pre-market authorisation requirements for food and colour additives, with the safety of dietary supplements and GRAS food ingredients regulated through post-market oversight mechanisms. Unsurprisingly, the FDC Act does not specifically refer either to the use of nanotechnologies as a process or the inclusion of nanoscale ingredients in foods or FCMs. However, as noted by the FDA Taskforce itself, the general nature of the FDC Act and most of the subsidiary Act enforced by the FDA 'are general in nature by design . . . offering flexibility to accommodate products made with new technologies or containing new kinds of materials'.[41]

A food is defined under s.201(f) of the FDC Act to mean:

1. articles used for food or drink for man or other animals
2. chewing gum
3. articles used for components of any such article.

A dietary supplement is defined under s.201(ff) of the FDC Act to include:

1. a product (other than tobacco) intended to supplement the diet that bears or contains one or more of the following dietary ingredients: (A) a vitamin; (B) a mineral; (C) an herb or other botanical; (D) an amino acid; (E) a dietary substance for use by man to supplement the diet by increasing the total dietary intake; or (F) a concentrate, metabolite, constituent, extract, or combination of any ingredient described in clause (A), (B), (C), (D), or (E); . . .

The adequacy of the FDA's regulatory existing framework with regards to nanotechnologies across each of the product categories it regulates, including foods, has been assessed by Taylor[42] and the FDA Taskforce.[43] Taylor's analysis of the FDA's framework and resourcing led him to conclude that 'while the FDA has most of the legal tools it needs to regulate most of the products of nanotechnology, significant gaps in authority remain'.[44] In relation to food this included, for example, a lack of power to acquire information pertaining to new nanofoods.[42] In their opinion, the FDA believed that for product categories subject to pre-market approval, including food and colour additives, 'the agency's authorities are generally comprehensive';[45] the adequacy of the framework was not, in their view, altered by the use of nanotechnologies in processing or the presence of nanoscale ingredients. However, for products not subject to pre-marketing authorisation processes, including

whole foods, dietary supplements, and GRAS food ingredients, the Agency noted that its 'oversight capacity [was] less comprehensive'.[45] In recognition of the current lack of knowledge and regulatory challenges potentially posed by some categories and their incorporation of nanotechnologies, the FDA Taskforce made a number of recommendations pertaining to data requirements and regulation perspectives. Given the breadth of these two reports, this chapter does not provide an in-depth analysis of the framework but rather key aspects for ensuring food safety and their application to nanofoods are highlighted. The adequacy, or in their view, the inadequacies of the FDA's regulatory framework and nanofoods has also been highlighted by FOE (ref. 15, pp 21–24).

Pursuant to the Chapter IV of the FDC Act, whole foods, dietary supplements and GRAS food ingredients may, subject to a few exceptions, be marketed in the USA without being subjected to pre-market authorisation approval.[46] As provided by the FDC Act, foods falling into these categories may only be marketed within the USA if they are considered to be safe; this burden rests with the sponsor. These products will however fall within post-market oversight regimes. A manufacturer or distributor of a dietary supplement containing new ingredients (as defined by s.413(c) of the FDC Act, see below for the definition of 'new') is however required to submit pre-market notification to the FDA which includes 'any citation to published articles, which is the basis on which the manufacturer or distributor has concluded that a dietary supplement containing such dietary ingredient will reasonably be expected to be safe' (§6 Dietary Supplement Health and Education Act of 1994). Under the 'GRAS notification program', a manufacturer or distributor of a GRAS food ingredient may voluntarily submit pre-market notification information to the FDA.[47] (See also ref. 42, pp. 33–35, and ref. 43, p. 26, for more information on the GRAS system.) While these categories of foods will be subject to the regulatory framework set down under the FDC Act regardless of whether they do or do not incorporate nanotechnologies, it is reasonably foreseeable that the lack of pre-market approval requirements in relation to these food categories will result in some food products incorporating nanotechnologies being marketed in the US without the FDA's knowledge.

The definition of new: s.413(c) of the FDC Act defines a 'new dietary ingredient' to mean 'a dietary ingredient that was not marketed in the United States before October 15, 1994 and does not include any dietary ingredient which was marketed in the United States before October 15, 1994'.

10.3.2 Regulatory Aspects Relating to Nanoscale Food Additives

Chapter IV of the FDC Act establishes a pre-marketing authorisation framework for food additives not considered as GRAS, and colour additives. As defined by s.201(s) of the FDC Act, a food additive is a substance which is 'directly or indirectly' added to food, and includes for instance, preservatives, thickeners, flavours and flavour enhancers. A colour additive, as defined by the

FDC Act, is a substance which is capable, 'when added or applied to a food, drug, or cosmetic, or to the human body' . . . 'of imparting color thereto'.

- A food additive means, 'any substance the intended use of which results or may reasonably be expected to result, directly or indirectly, in its becoming a component or otherwise affecting the characteristics of any food' (s.201(s)).
- A colour additive, 'means a material which – (A) is a dye, pigment, or other substance made by a process of synthesis or similar artifice, or extracted, isolated, or otherwise derived, with or without intermediate or final change of identity, from a vegetable, animal, mineral, or other source, and (B) when added or applied to a food, drug, or cosmetic, or to the human body or any part thereof, is capable (alone or through reaction with other substance) of imparting color thereto; except that such term does not include any material which the Secretary, by regulation, determines is used (or intended to be used) solely for a purpose or purposes other than coloring. . .' (s.201(t)(1) FDC Act).

Food and colour additives may not be included in foods without having been subjected to a safety assessment, and authorised for use in food by the FDA (see 21 USC 348 and 21 USC 379e respectively). Pursuant to Chapter IV of the FDC Act, the safety of the substance must be reviewed and established by the FDA (as set out in s.409 FDC Act; see also Parts 170–180 of Title 21 Foods and Drugs of the Code of Federal Regulations) on the basis of data supplied to the Agency by the sponsor and a regulation must then be published by the Agency establishing the conditions under which the food additive may be legally used (see ss.401 and 409 FDC Act). As noted by Taylor, while 'the burden of proof is on the sponsor to prove safety, the FDA has full control over testing requirements, and the safety standard is strict – "reasonable certainty of no harm".[48] Moreover, as noted by the FDA Taskforce in their review, data supplied to the FDA as part of the authorisation process may include information on the physicochemical characteristics of the substance including particle size. This they note allows the regulator to 'place limitations on the physical and chemical properties of food additives, which include particle size'.[49] Once authorised, the food additive is included in the FDA's positive list (the 'Food Additives Status List') and may be subject to maximum permitted levels, and other restrictions.[50] Maximum permitted levels are based on the substance's mass, and as such, may not be appropriate for nanoscale food additives. However, unlike the Australian and New Zealand framework (discussed below), it would appear that the FDA is invested with the power to differentiate food additives on the basis of their particle size and could, in the event of safety concerns being raised in relation to food additives containing nanoscale ingredients, 'publish a proposed rule to amend the food additive regulation to address under what circumstances the nanoscale version of the substance may be safely used'.[49] Colour additives are subjected to a similar premarket approval process, and once authorised for use are included on a positive

list (the 'Color Additives Status List'),[51] along with any conditions for their use. (For a more detailed review of the pre-market authorisation process for colour additives, see ref. 43, pp. 26–27.) Importantly, as with food additives, pursuant to the FDC Act (see 721 FDC Act, and Part 71 (Color additive petitions) of Title 21 Foods and Drugs of the Code of Federal Regulations), the FDA may require data pertaining to particle size. As suggested by the FDA Taskforce, should concerns be raised in relation to the safety of food colourings containing nanoscale ingredients, the regulator would have the necessary legislative power to 'publish a proposed rule to amend the listing regulation to address under what circumstances the nanoscale version of the substance be safety used'.[52] This is a significant difference between the USA and, for example, Australia and New Zealand, as it provides the FDA with the necessary legislative scope to re-evaluate an authorised food additive or colour additive which raises safety concerns on the basis of its particle size.

10.3.3 Regulatory Aspects Relating to FCMs Produced by Nanotechnologies

The regulatory status of FCMs under the FDC Act is codified in Title 21 (Food and Drugs), Chapter 9. It is based on the general safety requirements for food, as described above. Based on these requirements, as implemented notably by the pre-market approval process, FCMs are also placed under the responsibility of the FDA. More specifically, the FDC Act prohibits the adulteration of food, and deems all substances that are intended, or may reasonably be expected, directly or indirectly, to become components of food or affect its characteristics, including FCMs, to be food additives. Food additives are automatically deemed to be unsafe and to cause food to be adulterated, unless the intended use of these substances is permitted under the FDA's food additive regulations, found in Title 21 of the Code of Federal Regulations (21 C.F.R.). Various exemptions from the FDA's pre-market approval requirement are included in the FDC Act as implemented by the FDA's regulations and case law, so that components that are not specifically listed as permitted for food contact applications in 21 C.F.R. may still be used in compliance with the FDC Act and the FDA's food additive regulations within the scope of these exemptions.

One of these exemptions is the so called 'No Migration' Exemption. This exemption is based on the definition of a food additive itself, which makes FCMs subject to the FDA's pre-market approval process only if they are intended, or may reasonably be expected, directly or indirectly, to become components of food or affect its characteristics. This has been interpreted by the courts, and accepted by the FDA, as requiring substances to migrate into food in more than insignificant amounts to be considered food additives.

Although there is no legal definition of what constitutes 'significant migration', there are sources of guidance that can be used as a basis to establish that a

component of a food contact application is not expected to migrate to food in more than insignificant amounts, and thus is not required to be specifically permitted under the FDA's food additive regulations to be legally used in food contact applications in the USA. One such source of guidance is the so-called 'Ramsey Proposal,' circulated by the FDA in 1969, which would have permitted the use, without the prior promulgation of an applicable food additive regulation, of substances that migrate to food in quantities no greater than 50 parts per billion (ppb). Named after its author, Dr. Lessel Ramsey, then Assistant Director of Regulatory Programs at the FDA's Bureau of Science, this regulation would have applied to all substances except those known to pose some special toxicological concerns, for example, a heavy metal, a known carcinogen, or a substance that produces toxic reactions at levels of 40 parts per million (ppm) or less in the diet of man or animals. Although never formally adopted by the FDA, the standards in the proposal are deemed scientifically acceptable. We note that this 50 ppb migration level is only justified to establish the FDA status of substances that are not known to raise any special toxicological concerns. A lower migration level would be recommended for substances with known or suspected toxicological properties.

Of course, the use of any substance in a food contact application remains first and foremost subject to the general safety requirement of the FDC Act, reinforced by the FDA's GMP regulation, 21 C.F.R. § 174.5(a)(2), which requires any food contact substance to be of a 'purity suitable for its intended use', and demands that the potential level of migration into food of any component of an FCM to be safe. Safety is defined in the food additive regulations as 'reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use' (see 21 C.F.R. § 170.3(i)). We note that while Section 409(c)(3)(A) of the FDC Act establishes that no food additive shall be deemed by the FDA to be 'safe' if the additive is found 'to induce cancer when ingested by man or animal, or if it is found, after tests which are appropriate for the evaluation of safety of [the additive], to induce cancer in man or animal', this general prohibition on the use of carcinogenic additives only applies to the 'substance that is actually intended for use in food or for food contact,' while all 'non-functional chemicals present in that substance would be called the 'constituents' of the additive' (see 21 C.F.R. § 170.3(i)). Such constituents include impurities, residual reactants, intermediates, manufacturing aids, and the products of side reactions and chemical degradation. Based on this distinction between the 'food additive' and its 'constituents', the FDA may permit the use of an additive that is non-carcinogenic if it contains 'safe' levels of carcinogenic constituents.

To evaluate the safety of carcinogenic impurities, the FDA has adopted risk assessment techniques whereby in most cases a risk is considered negligible, and the intended use of a substance to which the risk is associated can thus be considered to be safe, if the calculated upper-bound lifetime risk from all sources of exposure is less than one in one million. The daily dose which corresponds to this level of risk is often referred to as the 'virtually safe dose' (VSD). To account for several potential sources of exposure to the substance, it

is reasonable to conclude that a specific application is safe if it contributes no more than 10% of the VSD.

In order to highlight the scope of the current US Federal regulatory framework for food packaging products produced by nanotechnologies and the implications thereof, the Project on Emerging Nanotechnologies, and the Grocery Manufacturers Association, initiated a study which was designed specifically to 'build understanding of how the regulatory process would apply to nanotech food-packaging materials and to identify issues that need to be addressed to ensure the process works effectively'.[53] In order to determine how the system would operate when faced with nanoscale substances in food packaging, three high-level working groups[54] developed three hypothetical food packaging products which were subsequently used to evaluate the regulatory process. The case studies included: an 'active packaging product' with functionalised antimicrobial nanoparticles; a 'smart packaging product' which incorporated nanobiosensors; and a product for carbonated beverages with superior barrier properties.[55] Having noted the inherent complexities and challenges of the current regulatory regime, the study found that 'the most challenging issues related to how the scientific and technical criteria for evaluating the food safety aspects of ENMs [engineered nanoscale materials] in food packaging will apply, in light of their novel properties. The few legal or policy issues also stem from the science'.[56] The legal issues were found to include, for example: the applicability of existing clearance instruments, such as food contact notifications and other positive lists, to nanoscale versions of existing food contact substances (when such instruments do not expressly refer to particle size); the applicability of the GRAS framework for food packaging containing nanoparticles both now, and in the medium term; the applicability of food additive petitions for ensuring the safety of such products prior to the product's entry onto the market; and definitional issues as to what constitutes 'nanoscale' for the purposes of the regulatory framework.

The study highlighted not only the significant number of knowledge gaps, which are hampering regulatory and scientific efforts in the area, but also the number of regulatory ambiguities that exist within the framework. The value of such an exercise can be very valuable, as at this early stage such an endeavour provides government, regulators and industry with the insights as to what information and action is required on their part to ensure the safe and responsible commercialisation of such products. In this case, such lessons are not only relevant to the US context, but other jurisdictions grappling with similar questions and concerns.

10.4 Regulatory Review: Australia and New Zealand

10.4.1 General Food Safety and Consumer Health Protection

The Australian and New Zealand legislative instrument of most relevance to the regulation of nanoscale food ingredients, novel foods, nanoscale food

additives and contaminants and nanotechnology derived FCMs is the Australian and New Zealand Food Standards Code (the Food Standards Code).[57] For a comprehensive review of Australia's food regulatory framework and its applicability to nanotechnologies, see Ludlow.[58] The Food Standards Australia and New Zealand (FSANZ), a trans-Tasman statutory body, administer this Code. It is important to note that not all of the food standards that form the Food Standards Code have been uniformly adopted by Australia and New Zealand. For example, New Zealand has adopted their own standards for maximum residue limits and processing requirements for all foods, food hygiene issues and primary production of food.

While the Food Standards Code establishes uniform food standards for foods sold (as defined in s.3A of the Food Standards Australia New Zealand Act 1991, Cth), prepared or imported into the two countries in relation to, for example, labelling, novel foods, substances added to foods, and contaminants, enforcement and compliance of the standards rests with the Australian state and territory governments and the New Zealand government. The principle objective of the Food Standards Code is to ensure that foods which fall within the regulatory scope of the Code that are sold, prepared or imported into Australia or New Zealand are safe. While the Code itself does not specifically refer to the use of nanotechnology, or the inclusion of nanoscale components in food, its inclusive and wide-ranging nature ensures that nanofoods and FCMs incorporating nanomaterials will fall under its scope. Importantly, pursuant to the Foods Standards Code, foods which are not specifically regulated under its standards may be sold, prepared or imported into either country without being subject to pre-market authorisation approval. The implication of this, as noted by Ludlow, is that 'FSANZ may not be aware that nanomaterials [are] included in the food or other item[s]',[58] which are legally available in the two countries.

In order to strengthen its regulatory oversight over foods that contain nanoscale-derived ingredients or additives, FSANZ in October 2008 released a document outlining several proposed amendments to Part 3 of the Food Application Handbook.[59] The amendments apply to three main areas of food production required to supply information to the regulator on, for example:

- 'the identity and purity of substances in applications for food additives, processing aids, nutritive substances and novel foods is adequate to properly define and assess the chemical entity for which approval is sought' (this would include information pertaining to particle size)
- 'information on the chemical and physical properties of substances in applications for food additives, processing aids, nutritive substances and novel foods is adequate to properly define and assess the application,' including particle size and
- 'information on particle size and morphology in cases where these characteristics may relate to the toxicity of a food contaminant' (FSANZ Application Handbook – Amendment No. 2 – 2008, p. 1).

These amendments arguably suggest that the regulator itself is concerned by the current lack of detailed information pertaining to, for example, nanoscale additives and processing aids, and the need to address knowledge gaps relating to potential toxicity.

As with the EU and US frameworks, the Food Standards Code provides for a mandatory pre-market approval system for certain foods; within Australia, categories subject to pre-market authorisation include novel foods, substances added to foods (including additives, vitamins and minerals and processing aids), and contaminants (including FCMs). These foods may not be sold or imported legally into Australia or New Zealand without having been subjected to a safety assessment, and authorised for use in food by the FSANZ. Pursuant to the Foods Standards Code, a novel food (as defined by Clause 1, Standard 1.5.1) may not be sold in Australia or New Zealand until a safety assessment, undertaken in accordance with FSANZ's guidelines, has been carried out on the novel food and the novel food has been expressly listed in Standard 1.5.1, along with any conditions of use. The categories of novel foods that may be relevant to nanotechnology include a non-traditional food which requires an assessment 'having regard to – . . . b) the composition or structure of the food; or c) the process by which the food has been prepared' (Clause 1, Standard 1.5.1 Food Standards Code). An important caveat, however, as noted by Ludlow, is that for a food to be considered a novel food it must also give rise to safety concerns.[59] As with the EU's current regulatory framework on novel foods and novel food ingredients (EC 258/97), it would therefore appear that while nano-structured foods could theoretically be considered as novel foods under the Code, the nano-structured food would have to be considered 'novel' (on the basis of its composition, structure or by virtue of the process by which it is prepared) *and* the properties of the nanofood would have to be considered to be substantially different to that of its macro-scale counterpart.

10.4.2 Regulatory Aspects Relating to Nanoscale Food Ingredients

Within Australia and New Zealand, substances added to food, including food additives, vitamins and minerals and food processing aids are similarly regulated by Part 1.3 of the Food Standards Code.

Standard 1.3.1 defines a food additive as 'any substance not normally consumed as a food in itself and not normally used as an ingredient of food, but which is intentionally added to a food to achieve one or more of the technological functions specified in Schedule 5. It or its by-products may remain in the food. Food additives are distinguishable from processing aids (see Standard 1.3.3) and vitamins and minerals added to food for nutritional purposes (see Standard 1.3.2).'

A food processing aid is defined by Standard 1.3.3 as 'a substance listed in Clauses 3 to 18, where – (a) the substance is used in the processing of raw materials, foods or ingredients, to fulfil a technological purpose relating to

treatment or processing, but does not perform a technological function in the final food; and (b) the substance is used in the course of manufacture of a food at the lowest level necessary to achieve a function in the processing of that food, irrespective of any maximum permitted level specified'.

As with the EU's new system for controlling food additives, food flavourings and food enzymes, the Code's standards are based on the principle that only substances that are explicitly authorised by the FSANZ may be added to foods. Pursuant to Part 1.3 of the Code, before the FSANZ may authorise, for example, an additive or a vitamin to be added to a food, the substance must be evaluated for safety and expressly incorporated into the Code (see Standard 1.3.1 (food additives) and 1.3.2 (vitamins and minerals) Food Standards Code). Authorised substances included in the Code's positive lists may also be subject to maximum permitted levels. However, with maximum permitted levels based on the substance's mass, Ludlow has expressed concerns over the appropriateness of this metric as a condition of use for some substances. In Ludlow's view, mass is unlikely 'to be an appropriate trigger if that additive is in a nanoform and therefore less material can be included to produce the same or change outcomes'.[59] Moreover, given the nature of the positive list, it would appear that the framework does not differentiate between nanoscale substances added to food and macro-scale substances which have been previously assessed for their safety and have already been explicitly authorised as substances that may be added to food.

10.4.3 Regulatory Aspects Relating to FCMs Produced by Nanotechnologies

Standard 1.4.3 of the Food Standards Code is the primarily standard for governing the composition, properties and use of articles and materials that may be in contact with food. Metal and non-metal contaminants and natural toxicants are also regulated under Standard 1.4.1. While Standard 1.4.1 does establish a positive list for some contaminants and natural toxicants, the purpose of the list is to set down, where possible, the maximum level for some contaminants and natural toxicants. As with the regulatory framework set out in the Code for substances added to foods, the maximum level is set by reference to the substance's mass. However, unlike the positive list established for substances added to foods, the list established under Standard 1.4.1 is not underpinned by the principle that only contaminants or natural toxicants explicitly authorised may be found in foods; rather, as noted by Ludlow (2007), 'if a contaminant is not listed, it is permitted' (at p. 14).[58]

The principle underlying this standard is that any article or material 'including packaging material, which may enclose materials such as moisture absorbers, mould inhibitors, oxygen absorbers, promotional materials, writing or other graphics' (Clause 1, Standard 1.4.3 Food Standards Code) intended to come into contact directly or indirectly with food must not 'cause bodily harm, distress or discomfort' (Clause 2(b), Standard 1.4.3 Food Standards Code). As

with the EU's Regulation (EC) 1935/2004, this Standard applies to a wide range of articles and materials that may come in contact with food, with its primary focus on ensuring the safety of these articles and materials. In doing so, the general safety requirement does not deal specifically with substances on their own, or in the articles or materials. This approach, which does not rely on 'positive' or 'negative' lists of additives, is also consistent to that adopted in jurisdictions such as Malaysia (pursuant to the Food Act 1983 and the Food Regulation 1985) and South Korea (pursuant to the Food Sanitation Act). Accordingly, as with EC 1935/2004, the Food Standards Code will only prohibit the use of a substance, regardless of its size, when it can be shown that the substance is 'likely' to cause harm to human health.[59] Within Australia, this general standard is supplemented by a more detailed standard on plastic materials for food contact use.[60] Unlike the EU, more detailed regulations have not been adopted for other types of articles or materials that may come in contact with food.

10.5 Discussion and Conclusions

As highlighted by this review, and eloquently stated by Taylor in respect to the USA, 'the regulatory system for food packaging is extraordinarily complex, legally and scientifically'.[53] This statement would appear to be equally true in relation to the EU regulatory framework for both food and FCMs. The findings of this review reiterate reports by, for example, the FSA, Chaudhry *et al.*, Taylor, and Ludlow, in that the current regulatory frameworks for food and food contact materials within jurisdictions such as the EU, the USA and Australia, are broad enough to 'catch' foods and FCMs which incorporate nanotechnologies. While the current frameworks are not designed to cope explicitly with the new challenges posed by the use of nanotechnologies, all the regulatory layers aimed at controlling the risks in the food chain and the general safety requirements which put the burden on the manufacturer and importer to only produce and place on the market food which is safe for consumption, should be effective in safeguarding against the irresponsible application of nanotechnology. Short of nano-specific regulatory requirements with, for example, a clear definition that encompasses the distinctive properties of nano-ingredients and additives, a clearly defined responsibility/liability for relevant products and applications, and appropriate permissible limits that relate to the (potential) effects of nano substances in food or an exclusive pre-market approval system, it is the case-by-case determination of the safety of the intended applications by the manufacturers which should guarantee that only safe applications of this new technology access the market. The recast of key regulatory instruments such as Regulation 258/97 (the Novel Foods Regulation) would appear to provide the EU with a unique opportunity to clarify some of the current ambiguities that exist in relation to, for example, how nanotechnology and nanoscience are defined, and pre-market safety evaluation of nanofoods. The recast of the Regulation, as with any

amendments to other relevant EU regulatory instruments will, however, need to be negotiated at the EU level, while taking into account other international frameworks to develop a harmonised strategy for the governance of nanotechnology risks.

One of the pertinent issues to have been highlighted in this chapter was in relation to the EU's current approach to regulating FCMs produced by nanotechnologies. As outlined above, while the current regulatory system might in principle be able to cope with the necessary safety guarantees for new nanomaterials, there are fundamental issues which could *de facto* block the authorisation of safe nanomaterials in food contact applications while not exclude the potential use of possibly untested nano forms of their bulk equivalents. Indeed, pursuant to the regulatory requirements for food contact, applications made entirely of plastic materials (monomers and food additives) are regulated by a positive list based system. Substances listed can be used by anyone, provided the potential restrictions and specifications are met. This then implies that the restrictions established are suitable to serve all potential uses, as long as a substance is listed, if *a priori* will have satisfied the requirements of the regulation. As authorisation for a nanomaterial on the basis of such an open-ended way is probably scientifically very difficult, this might *de facto* block the listing of nanomaterials on the positive lists. In contrast, a regulatory structure similar to that applied by the FDA would provide the framework for a case-by-case review of each nanomaterial application, which on the basis of Article 3 criteria should be safe.

Although there is not enough scientific knowledge at present to warrant application of the precautionary principle to nanofood, it seems beneficial for the food industry to develop appropriate initiatives to self-regulate and test those nanotechnology applications that may carry a relatively greater risk to consumers. Given the potential and interest in the use of nanotechnologies in food products, it would appear beneficial for all parties to take a proactive and transparent approach to ensure safe use of the technology in food products.

References

1. P. Phillips, in *Agricultural Globalization, Trade and the Environment,* ed. C. Moss, G. Rausser, A. Schmitz, T. Taylor and D. Zilberman, Kluwer, Boston, 2002, p. 504.
2. Q. Chaudhry, M. Scotter, J. Blackburn, B. Ross, A. Boxall, L. Castle, R. Aitken and R. Watkins, *Food Addit. Contam.*, 2008, **25**(3), 241–258.
3. European Food and Safety Authority, *Draft Scientific Opinion: Draft Opinion of the Scientific Committee on the Potential Risks Arising from Nanoscience and Nanotechnologies in Food and Feed Safety*, EFSA, Brussels, 2008.
4. Helmut Kaiser, *Study: Nanotechnology in Food and Food Processing Industry Wide 2003-2006-2010-2015*, Helmut Kaiser, Tubingen, 2004.

5. Cientifica, *Nanotechnologies in the Food Industry*, Cientifica, London, 2006.
6. C. I. Moraru, C. P. Panchapakesan, O. Huang, P. Takhistov, S. Liu and J. L. Kokini, *Food Technol.*, 2003, **57**(12), 24–29.
7. K. C. Arabe, *Industrial Market Trends*, 5 September 2002, available at: www.news.thmoasnet.com.
8. M. Lallo, The Age, 11 March 2008, available at: http://www.theage.com.au/news/epicure/sweating-the-small-stuff/2008/03/10/1204998320564.html.
9. ETC Group, *Down on the Farm, ETC Group*, Ottawa, 2004.
10. Organic Consumers Organisation, *Organic* View, 2006, Autumn, 7.
11. K. Lyons, *Chain Reaction*, June 2006, 38–39.
12. Soil Association, *Press Release: Soil Association first organisation in the world to ban nanoparticles – potentially toxic beauty products that get right under your sk*in, 17 January 2008.
13. International Union of Food, Farm and Hotel Workers, *Resolution on Nanotechnolog*y, passed by International Union of Food, Farm and Hotel Workers at the 25th IUF Congress meeting in Geneva, March 19–22, 2007.
14. J. Paull and K. Lyons, *J. Org. Systems*, 2008, **3**(1), 3–22.
15. G. Miller and R. Senjen, *Out of the Laboratory and on To Our Plates: Nanotechnology in Food & Agriculture*, Friends of the Earth Australia, Europe and US, Melbourne, 2008.
16. A. Salleh, *ABC Science Online*, 11 March 2008, available at: http://www.abc.net.au/news/stories/2008/03/11/2186232.htm.
17. L. Dayton and S. Ryan, *The Australian*, 11 March 2008, available at: http://www.theaustralian.news.com.au/story/0,25197,23353574-30417,00.html.
18. M. Berger, *Nanowerk*, 16 April 2008, available at: http://www.nanowerk.com/spotlight/spotid=5305.php.
19. S. Poulter, *The Daily Mail*, 11 March 2008, available at: http://www.dailymail.co.uk/news/article-531128/104-products-shelves-contain-toxic-grey-goo-stealth-say-Friends-Earth.html.
20. Azonano.com, *Azonano.com*, 11 March 2008, available at: http://www.azonano.com/news.asp?newsID=6057.
21. G. Miller and R. Senjen, *Out of the Laboratory and on To Our Plates: Nanotechnology in Food & Agriculture*, Friends of the Earth Australia, Europe and US, Melbourne, 2008, p. 3.
22. Swiss Retailers' Association, *Code of Conduct: Nanotechnologies*, IG DHS, Geneva, 2008.
23. M. W. Bauer and G. Gaskell, (Eds.), *Biotechnology: The Making of a Global Controversy,* Cambridge University Press, London, 2002.
24. T. Bernauer, *Genes, Trade and Regulation*: *The Seeds of Conflict in Food Biotechnology*, Princeton University Press, Princeton, 2003.
25. Food Standards Agency, *Draft Report of the FSA Regulatory Review: A Review of the Potential Implications of Nanotechnologies for Regulations*

and Risk Assessment in Relation to Food, Food Standards Agency, London, 2006, p. 2.
26. Food and Drug Administration, *Nanotechnology-A Report of the U.S. Food and Drug Administration Nanotechnology Task Force*. FDA, Washington DC, 2007, p. ii.
27. European Commission, *Regulatory Aspects of Nanomaterials: Summary of legislation in relation to health, safety and environment aspects of nanomaterials, regulatory research needs and related measures {COM(2008) 366 final}*, Commission of the European Communities, Brussels, 2008, pp. 21–22.
28. European Commission, *Proposal for a Regulation of the European Parliament and of the Council on novel foods and amending Regulation (EC) No XXX/XXX [common procedure]*, *COM(2007) 872 Final*, Brussels, 14 January 2008, p. 2.
29. Article 3(2)(a)(iii) 2008/0002 (COD) *Proposal for a Regulation of the European Parliament and of the Council on novel foods and amending Regulation (EC) No XXX/XXX [common procedure]*.
30. Preamble 6 2008/0002 (COD) *Proposal for a Regulation of the European Parliament and of the Council on novel foods and amending Regulation (EC) No XXX/XXX [common procedure]*.
31. European Commission, *Proposal for a Regulation of the European Parliament and of the Council on novel foods and amending Regulation (EC) No XXX/XXX [common procedure], COM(2007) 872 Final*, Brussels, 14 January 2008.
32. G. Miller and R. Senjen, *Out of the Laboratory and on To Our Plates: Nanotechnology in Food & Agriculture*, Friends of the Earth Australia, Europe and US, Melbourne, 2008, p. 40.
33. European Commission, *Amended Proposal for a Regulation of the European Parliament and of the Council establishing a common authorisation procedure for food additives, food enzymes and food flavourings, COM(2007) 672 Fi*nal, 24 October, Commission of the European Communities, Brussels, 2007, p. 3.
34. The provisional list of additives, which was made publicly available on 11 April 2008, can be accessed from DG SANCO's website at the following address: http://ec.europa.eu/food/food/chemicalsafety/foodcontact/documents_en.htm.
35. EFSA, *Call for Scientific Data on Applications of Nanotechnology and Nanomaterials used in Food and Feed*, 31 January 2008. Available at: http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178680756675.htm.
36. EFSA Opinion on 'The Potential Risks Arising from Nanoscience and Nanotechnologies on Food and Feed Safety', adopted 10 February 2009, available at: www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902361968.htm.
37. European Food and Safety Authority, *Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request related to a 14th list of substances for food contact*

materials Question No EFSA-Q-2006-1, EFSA-Q-2006-178, EFSA-Q-2006-116, EFSA, Brussels.
38. European Food and Safety Authority, *Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request related to a 14th list of substances for food contact materials Question No EFSA-Q-2006-1, EFSA-Q-2006-178, EFSA-Q-2006-116*, EFSA, Brussels, p. 2.
39. European Food and Safety Authority, *Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request related to a 14th list of substances for food contact materials Question No EFSA-Q-2006-1, EFSA-Q-2006-178, EFSA-Q-2006-116*, EFSA, Brussels, p. 4.
40. European Food and Safety Authority, *Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request related to a 14th list of substances for food contact materials Question No EFSA-Q-2006-1, EFSA-Q-2006-178, EFSA-Q-2006-116*, EFSA, Brussels, p. 3.
41. Food and Drug Administration, *Nanotechnology-A Report of the U.S. Food and Drug Administration Nanotechnology Task Force*. FDA, Washington DC, 2007, p. 4.
42. M. R. Taylor, *Regulating the Products of Nanotechnology: Does FDA Have the Tools It Needs?*, Woodrow Wilson International Center for Scholars, Washington DC, 2006.
43. Food and Drug Administration, *Nanotechnology-A Report of the U.S. Food and Drug Administration Nanotechnology Task Force*. FDA, Washington DC, 2007.
44. M. R. Taylor, *Regulating the Products of Nanotechnology: Does FDA Have the Tools It Needs?,* Woodrow Wilson International Center for Scholars, Washington DC, 2006, p. 51.
45. Food and Drug Administration, *Nanotechnology-A Report of the U.S. Food and Drug Administration Nanotechnology Task Force*. FDA, Washington DC, 2007, p. iii.
46. M. R. Taylor, *Regulating the Products of Nanotechnology: Does FDA Have the Tools It Needs?,* Woodrow Wilson International Center for Scholars, Washington DC, 2006, p. 30.
47. See http://www.cfsan.fda.gov/~dms/opa-noti.html.
48. M. R. Taylor, *Regulating the Products of Nanotechnology: Does FDA Have the Tools It Needs?,* Woodrow Wilson International Center for Scholars, Washington DC, 2006, p. 36.
49. Food and Drug Administration, *Nanotechnology-A Report of the U.S. Food and Drug Administration Nanotechnology Task Force*. FDA, Washington DC, 2007, p. 26.
50. See http://www.cfsan.fda.gov/~dms/opa-appa.html#ftnD.
51. See http://www.cfsan.fda.gov/~dms/opa-appc.html.

52. Food and Drug Administration, *Nanotechnology-A Report of the U.S. Food and Drug Administration Nanotechnology Task Force*. FDA, Washington DC, 2007, p. 27.
53. M. R. Taylor, *Assuring the Safety of Nanomaterials in Food Packaging: The Regulatory Process and Key Issues,* Woodrow Wilson International Center for Scholars, Washington DC, 2008, p. 5.
54. As noted by Taylor (2008), the three working groups were structured around the following topics: 1) Law, Policy and Process; 2) Science; and 3) Industry Stewardship.
55. For a description of the three case studies, Appendix B, M. R. Taylor, *Assuring the Safety of Nanomaterials in Food Packaging: The Regulatory Process and Key Issues*, Woodrow Wilson International Center for Scholars, Washington DC, pp. 57–59.
56. M. R. Taylor, *Assuring the Safety of Nanomaterials in Food Packaging: The Regulatory Process and Key Issues,* Woodrow Wilson International Center for Scholars, Washington DC, 2008, p. 6.
57. Available at: http://www.foodstandards.gov.au/thecode/foodstandardscode.cfm.
58. K. Ludlow, *Tasmania Law Revi*ew, 2007.
59. FSANZ Application Handbook – Amendment No. 2 – 2008.
60. See Australian Standard 2070-1999 Plastics materials for food contact use. For more information on this standard, see ref. 58.

CHAPTER 11

An Outline Framework for the Governance for Risks of Nanotechnologies in Food

MARTIN MÖLLER,[a] ULRIKE EBERLE,[b] ANDREAS HERMANN[c] AND CLAUDIA SOM[d]

[a] Öko-Institut e.V., Merzhauser Straße, D-79100, Freiburg, Germany; [b] Corsus – Corporate Sustainability, Behringstraße 28a, D-22765, Hamburg, Germany; [c] Öko-Institut e.V., Rheinstraße 95, D-64295, Darmstadt, Germany; [d] EMPA, Lerchenfeldstrasse 5, CH-9014, St. Gallen, Switzerland

11.1 Introduction

At present, the contribution of nanotechnologies to sustainable nutrition, which is environmentally friendly, constitutional and ethically responsible,[1] is estimated to be rather low. In perspective, however, applications such as the enrichment of food with engineered nanomaterials (e.g. iron) could generate a constitutional advantage in developing countries to reduce malnutrition problems. A requirement for this is the eco- and human-toxicological safety of the applied nanomaterials, and that the products are affordable by those people who suffer from malnutrition. Moreover, food packaging with engineered nanomaterials already offers several benefits for the consumers and holds a lot of potential for the future. Nanotechnology-derived food packaging can also reduce the environmental impacts. However, the technology needs to fulfil certain requirements, such as the absence of migration of nanomaterials into

RSC Nanoscience & Nanotechnology No. 14
Nanotechnologies in Food
Edited by Qasim Chaudhry, Laurence Castle and Richard Watkins

Published by the Royal Society of Chemistry, www.rsc.org

the contained food, and that it is safe to the health of the consumer and the environment.[2]

Against this background, the main challenge is not only aiming at the short-term achievable benefits, but also towards a more sustainable nutrition with minimal possible health and eco-toxicological risks of the engineered nanomaterials in food. Thus, a risk governance framework for engineered nanomaterials in food and food packaging is required, which can facilitate and promote the implementation of sustainability potentials, but at the same time avoids possible risks to people and the environment. An approach like this needs to be based on the precautionary principle as well as life cycle perspective, and should also contain binding procedures in relation to stakeholder involvement for all players. These guiding principles for a risk government framework are explained in Section 11.2 in general and are applied as the basis for recommendations for a sustainable use of engineered nanomaterials in the food sector in Section 11.3.

Knowledge about nanotechnologies is expanding rapidly, especially regarding new applications. Many questions regarding the ecotoxicological impact of nanomaterials on humans and the environment, however, are unanswered at this stage and need further research. Nanomaterials are a highly diverse group. Thus, some of these uncertainties are unlikely to be resolved in the short-term future. The precautionary principle gives guidelines on how to deal with these uncertainties. In order to achieve a holistic view on the risks to human health and the environment, the entire life cycle of nanomaterials and nanoproducts needs to be addressed. This includes the possible release of nanoparticles from nanoproducts throughout all stages of the product life cycle, and the ultimate fate of the nanoparticles on re-use, recycling or disposal. A crucial task in this regard is to classify nanomaterials according to their (potential) risk, during which process risk communication must be as transparent as possible (cf. Section 11.3.1).

Based on the results of risk analysis and safety research, the existing statutory provisions and incentive instruments need to be reviewed and adapted if necessary, to ensure that nanomaterials are handled and used responsibly (cf. Section 11.3.2).

Regulatory measures have to be flanked by a consequent perception of corporate responsibility and product stewardship on the part of the producers. This especially incorporates risk minimisation when developing and producing nanoproducts, and calls for design of the products with an aim to ensure safety and sustainability (cf. Section 11.3.3).

Moreover, the responsible and successful introduction of new technologies demands a high level of transparency in risk regulation and risk communication. Participatory processes are an important component here, meaning that all relevant stakeholders must be involved. An appropriate method of systematically exploring and analysing more complex effects which arise in practice is to conduct the participatory processes in tandem with the development of nanotechnology applications in the food sector (cf. Chapter 11.3.4).

11.2 Guiding Principles for Risk Governance

The three principles explained in the following sections have to be the basis for action and 'guiding' for the governance of risks. Risk governance itself is the regulation process of risks which consists of preliminary proceedings (such as screening, ranking, scoping), risk assessment, risk evaluation, risk management and risk communication. Standard risk regulation has to fulfil special guidelines that have been developed by various commissions, e.g. the risk assessment working group of DG Health and Consumers;[3] the German ad hoc risk commission[4] and the working group on harmonisation of risk assessment procedures of the European Commission (EU Commission 2000, communication from the Commission on the precautionary priciple, COM(2000)1, Brussels, 02.02.2000, http://ec.europa.eu/dgs/health_consumer/library/pub/pub07_en.pdf).

The three guiding principles for risk governance are:

- the *precautionary principle*, which is the main action-conducting principle for sustainable development incorporating a temporal perspective[5]
- *life-cycle thinking*, which includes a holistic perspective and
- *stakeholder involvement*.

This means that risk governance:

- has to take into account today the risks for future generations to fulfil their needs with the target to avoid or minimise these risks
- it has to consider risks along the whole value chain of products or services from extraction of resources to disposal of products at the end of their useful service life (i.e. from 'cradle to grave') and
- has to assure an adequate participation of all relevant societal groups and has to include trans-disciplinary perspectives on risks.

11.2.1 The Precautionary Principle

The precautionary principle is acknowledged at international and European levels. With the treaty of the European Union in 1992, the precautionary principle was established at the EU level for environmental policy:

Community policy on the environment [. . .] shall be based on the precautionary principle and on the principles that preventive action should be taken, that environmental damage should as a priority be rectified at source and that the polluter should pay. (Official Journal C 191, 29 July 1992, Art. 130 r, para. 2).

In EU food law it is explicitly mentioned as a basic principle (Article 7 of Regulation (EC) No. 178/2002). Article 7 (1) of that Regulation specifies that

in specific circumstances where, following an assessment of available information, the possibility of harmful effects on health is identified but scientific uncertainty

persists, provisional risk management measures necessary to ensure the high level of health protection chosen in the Community may be adopted, pending further scientific information for a more comprehensive risk assessment.

The legislative framework for the application of the precautionary principle in the EU has been fixed by the European Court of Justice (ECJ) in numerous rulings. In this respect, the precautionary principle in practice is to be applied especially in those cases where – on the basis of an objective scientific assessment – there is a reason to have concerns that risks to the environment as well as to human, animal or plant health could be unacceptable or incompatible with the high level of protection chosen in the EU. Hence, should there be a threat of irreversible and severe harm to human, animal and plant health, as well as to the environment, the non-availability of a proven link between cause and effect, or of a proof of the magnitude of risks related to a product or process, can not be used as an argument to delay the necessary measures.

Nevertheless, the precautionary measures cannot be issued on a completely groundless basis. Rather, the application of the precautionary principle has to be based on first scientific references concerning any serious or irreversible possible detriments, or on a scientifically plausible risk hypothesis.

The jurisdiction of the ECJ and the Communication of the Commission (COM(2000) 1 final) led to a normative framework (basic principles) for the risk management in general and for the application of the precautionary principle specifically. This could also be used as an example for regulation. When applying the precautionary principle, the following basic principles have to be taken into account:

- proportionality
- non-discrimination
- consistency
- examination of the benefits and costs of action or lack of action and
- examination of scientific developments.

With regard to the recommendations given on regulatory aspects in Section 11.3.2, selected basic principles are discussed in more detail below.

11.2.1.1 Proportionality

Measures that are based on the precautionary principle have to allow for an appropriate level of protection for health and the environment but must not be disproportionate to the desired level of protection and must not aim at zero risk. In this respect, all possible alternatives for the choice of risk management measures should be evaluated. Thus, measures that ensure an appropriate level of protection and at the same time impose the least possible restrictions are to be preferred. In the context of proportionality, the possibility to replace products or processes with other less risky products and processes should be analysed as an alternative.

On the basis of the precautionary principle, banning the production or use of engineered nanomaterials as the most outreaching measure is not *per se* disproportionate. Rather, a ban can in one case be a disproportionate measure with regard to the potential risk, but in another case it can be the only possible measure to ensure the high level of protection chosen. When judging on the proportionality of measures, long-term risks also have to be taken into account.

11.2.1.2 Examination of the Benefits and Costs of Action or Lack of Action

The goal of precautionary measures is to reduce risks up to a reasonable level. In this respect, the EU Commission affirms that 'requirements linked to the protection of public health should undoubtedly be given greater weight than economic considerations'. Pros and cons related to action and non-action have to be examined before taking measures. For that purpose, an economic cost–benefit analysis has to be carried out, provided that it is appropriate and feasible.

11.2.1.3 Examination of Scientific Developments

When decisions are made in the context of the precautionary principle, a certain amount of scientific uncertainty will always remain with respect to the existence or the amplitude of environmental and health risks. It is therefore important to request further scientific research as well as to generate and evaluate new scientific data.

Subsequently, measures based on the precautionary principle shall be re-examined and if necessary modified depending on new scientific results. The decisive factor for the amendment respectively or the repeal of the measure should not be the time factor but rather scientific findings. Since research should be carried out with the aim to allow a better and more complete evaluation of risks, it is also important that the measures are subject to regular scientific monitoring.

11.2.2 Life Cycle Perspective

In general, industrial systems are characterised by interdependent processes and activities, requiring a systems approach for considering technology from a holistic perspective, especially when it comes to assessing and managing the risks.

Life cycle thinking is such a holistic approach for the assessment of environmental and health effects associated with a product, process or activity, which is derived from the conceptual framework of life cycle assessment (LCA). Every life cycle of a product or service starts with the extraction and processing of raw materials, followed by manufacturing, transportation and use, and ends with waste management including recycling and final disposal. Therefore, life cycle thinking is often also referred to as a 'cradle to grave' approach. As each of the life cycle stages is connected with the consumption of resources and the

discharge of emissions, a number of different environmental and health impacts have to be considered. By using life cycle thinking, key impacts throughout all life cycle stages of a system can be identified. Moreover, this approach also helps to discover trade-offs and problem shifting both between different life cycle stages (e.g. shift of environmental burden from the production into the use phase) and between different environmental aspects/media (e.g. CO_2 emissions and hazardous substances).

In contrast to this holistic perspective, the current practice of risk assessment and risk management of many enabling technologies (such as nanotechnologies) is characterised by a limited perspective, often not exceeding the 'cradle to gate' point of view and especially leaving out aspects at the end of the products' service life, such as waste water treatment, recycling processes and final disposal. Thus, if it is aimed to gain momentum towards sustainable production and consumption that respects the carrying capacity of the ecosystems of the planet, life cycle thinking should provoke greater awareness both in industry/business and public administration.

Within this perspective, business-oriented or facility-based life cycle thinking should encompass the following three different dimensions.[6]

- Inside the facility (within the 'gate'): at this level, life cycle thinking aims to evaluate alternative materials or to modify processes in order to reduce the energy or resource demand of the facility as well as to reduce the exposition to the employees and the environment.
- Upstream (towards 'cradle'): life cycle thinking in the upstream perspective focuses on the evaluation and modification of the supply chain management in order to replace hazardous substances, reduce the hazardous potential of a used substance or increase the sustainability performance of the supplier(s).
- Use and end-of-life ('gate to grave'): within the downstream perspective, the use stage is evaluated and optimised (e.g. reduce energy demand, extend useful service life, reduce exposition to customers) and also the impact on end-of-life services (like waste water treatment, recycling, waste incineration) is systematically taken into account.

Besides facilitating decision making at the business level, life cycle thinking can also help substantially towards more coherent and less complex policy making. Examples for life cycle thinking in EU policies include the Integrated Product Policy Communication (2003/302/EC), the two Thematic Strategies on the Sustainable Use of Natural Resources (2005/670/EC), the Prevention and Recycling of Waste (2005/666/EC), the Directive for Energy-using Products (2005/32/EC) and the Action Plan on Sustainable Consumption and Production (2008/2110/EC).

Appropriate tools for implementing life cycle thinking are Life Cycle Assessment as standardised within ISO 14040 series (cf. ISO 14040 and ISO 14044),[7,8] Life Cycle Costing (LCC), Design for the Environment (DfE) and Product Sustainability Assessment (PROSA,[9] and www.prosa.org).

Key success factors for studies based on life cycle thinking are a product-type-specific approach, built upon a harmonised and scientifically robust methodological basis, as well as the availability and quality of life cycle inventory data. However, even if major data gaps exist, the life cycle thinking approach helps to identify and prioritise aspects where risk assessment efforts have to be increased.

11.2.3 Stakeholder Involvement

The aim of risk regulation is to take measures that are broadly accepted. Thus, it is indispensable to include into decision making the different opinions that exist in a society on how risky a technology is. This is especially relevant because no common understanding exists for the definition of risk.[10] Depending on the scientific discipline or the context of the stakeholders, risks are defined in a different manner and thus the suggestions on how to avoid or minimise one and the same risk also differ significantly. Under a technical-scientific perspective, risk is the product of probability of occurrence and extent of damage. In contrast to this, epidemiology knows the relative risk of one population compared to another, a risk which is not determinable or which is characterised by insecurity or lack of knowledge. Furthermore, within the sociological perspective, risks are negative implications, which are context-sensitive and are matter of subjective perceptions. As risk perception depends on individual, social or societal aspects and differs from one individual to another, also risk evaluation will differ individually.[5]

Therefore. a broadly accepted risk governance requires involving all those different perspectives. However, not only the different scientific opinions have to be taken into account, but also subjective perceptions of different societal groups have to be considered. The German ad hoc risk commission[4] therefore calls for involving health aspects as well as environmental, economic, technical, social and political aspects for development of measures and evaluates different options for action against health, environmental, economic, technical and social criteria. Putting this into practice requires the involvement of the relevant stakeholders into the risk regulation process, and that at different stages of risk regulation:[4]

For risk assessment, the different disciplinary scientific perspectives have to be integrated and balanced. In doing so, the assessment needs to be based on objective facts (e.g. identification of possible damages, exposition estimation, dose–effect relationship) and the assessments based on normative values should be strictly separated and marked as such. Results from risk assessments should be the identification of qualitative and quantitative hazards, explanations on the assessment and indications on the certainty of the risk assessment. Thus, risk assessment requires involving stakeholders from different scientific disciplines.

In risk evaluation, the results of risk assessment will be discussed and evaluated regarding the consequences for risk management. At this stage of risk regulation, societal value categories will be included and the need for action will be mainly identified. This requires involving relevant stakeholders from science

as well as from societal groups, governmental and non-governmental organisations. Only with the involvement of the relevant stakeholders, is it possible to achieve a societal consensus on which risks are tolerable for a society and which are not, as well as on the desired level of protection, especially with respect to precautionary measures.

During risk management also the participation of societal groups, stakeholders and a broad section of the public is essential for the identification of options for measures, the analysis of possible implications of the identified measures and the final evaluation of the measures.

In addition to the obligatory need for involvement of all relevant stakeholders to allow a trans-disciplinary assessment, evaluation and regulation of risks, the risk regulation also requires a transparent and adequate risk communication (cf. Section 11.3.4).

11.3 Components of a Harmonised Risk Governance Approach in the Food Sector

The current situation regarding risk governance and risk communication in the food sector is characterised more or less by a cautious and reserved communication policy, especially when looking at the manufacturers of food containing engineered nanomaterials.[2] Occasionally, this behaviour is also referred to as a 'strategy of silence'.[11]

Several stakeholders, however, have recently increased transparency and have proactively developed voluntary codes of conduct. Besides the engagement in dialogues with other stakeholders, their main objectives are to set up a framework for the responsible production and use of nanomaterials in a broad sense, and to provide complementary measures for the regulatory instruments. Four of the most prominent examples for voluntary codes of conducts are discussed by Grobe *et al.*[11] These comprise:

- the Global Core Principles of Responsible Care developed by the International Council of Chemical Associations (ICCA)
- the Code of Conduct for Responsible Nanosciences of the European Commission
- the Responsible Nano Code of the Nanotechnology Industries Association (NIA)
- the Nano Risk Framework by the US-based NGO Environmental Defense.

All of these codes are based on a cross-sector approach and do not contain any specific information regarding the use of engineered nanomaterials in the food sector. Although food-related aspects are not addressed in detail, the first draft structures for framing the nanotechnology risks for risk assessment throughout the life cycle, as well as for risk management and communication strategies, have been outlined.

In 2008, a food-specific code of conduct was introduced by the Swiss Retailers Association (IG DHS), forcing their food and packaging suppliers to provide detailed information about the use of engineered nanomaterials.[12] Via questionnaire, the signing members of IG DHS (Co-op, Denner, Manor, Migros, Valora and Vögele) will request information from their suppliers on whether their food products or food packaging contain engineered nanomaterials or if engineered nanomaterials are used during the production process. This action was motivated by the negative experience gathered with genetically modified food in the past, and the desire for avoiding bad consumer publicity when it comes to nanotechnology.[13]

Despite the fact that some of the voluntary codes offer interesting benchmarks for responsible research, production and use of engineered nanomaterials, the different approaches remain isolated and lack consistency. With the multitude of codes already existing it has to be feared that industrial players might opt for the code with the most lenient provisions. Furthermore, most of the codes lack provisions to enforce action or compliance and only reflect public relation concerns.[11]

Against this background, the major components of a harmonised risk governance approach in the food sector are developed in the sections of this chapter that follow.

11.3.1 Focus on Safety Research

For risk assessment, it is essential to know the toxicological effects of the engineered nanomaterials that are intended for use in food products. Toxicological properties of substances depend on various different determinants. For nanomaterials – unlike their counterparts at the micro scale – in particular the property 'size' is a relevant factor. It is feared 'that the very properties that make nanomaterials so technologically attractive – such as their high reactivity and ability to penetrate barriers – could make them harmful to people or the environment'.[14] Their size allows nanomaterials to penetrate barriers structures in organisms, e.g. cell membranes. Thus, nanomaterials can penetrate cellular barriers in living organisms, but so far it is neither known what kind of effects they can induce in those cells nor is it known what happens with nanomaterials in other organs, if they were to translocate there. It is, however, important to point out that nanomaterials are not toxicologically hazardous *ipso facto*, and therefore need to be investigated on a case by case basis.

Toxicological safety of engineered nanomaterials is an indispensable precondition for the market launch of nanoproducts following the precautionary principle (cf. Section 11.2.1). Thus, research on the behaviour of engineered nanomaterials throughout their whole life cycle has to be intensified in order to address the existing data gaps and uncertainties regarding:

- migration of engineered nanomaterials from products/product systems, e.g. from food packaging into food, and from food (packaging) into the environment

- the behaviour and fate of engineered nanomaterials in food and the environment
- ADME (absorption, distribution, metabolism and excretion) behaviour of engineered nanomaterials within the body and their effects and impact on living organisms.

Current discussions on safety aspects of engineered nanomaterials have tended to focus mainly on production and applications. As experience from many other fields has shown, the increasing use of substances also raises questions related to end-of-the-life treatments, such as recycling or disposal. Therefore, appropriate procedures must be implemented in time to avoid or minimise any risk that may occur at any stage of the products life cycle. This calls not only for research on increasing safety research concerning the production processes, or products and applications, but also for studies investigating the behaviour and fate of nanomaterials in waste streams and treatment processes. Regarding food contact materials and food packaging, particularly biological, mechanical and chemical recycling processes need to be taken into account. Risk assessment must also be based on the results of these investigations. Not only research on the behaviour of engineered nanomaterials within humans, products or the environment has to be intensified but also research concerning the development of testing and monitoring methods needs to be enforced. So far, no generally accepted testing method for nanomaterials exists, which would allow a standardised characterisation of engineered nanomaterials to provide a basis for a risk assessment.

Significant contributions to all the mentioned aspects can be expected from the Organisation for Economic Co-operation and Development (OECD) and its Working Party on Manufactured Nanomaterials (WPNM). With a focus on both consumer health and environmental protection, guidelines for the sustainable use of engineered nanomaterials are being developed. For example, OECD WPNM project group 2 is working on an international harmonised research strategy regarding health and environmental risks. Within project group 3, OECD experts are currently testing a set of 14 commercially relevant manufactured nanomaterials comprising single- and multi-walled carbon nanotubes, carbon black, fullerenes, aluminium oxide, cerium oxide, silicon dioxide, titanium dioxide, zinc oxide, iron, silver, polystyrole, dendrimere and nanoclays. Moreover, project group 4 covers the adaptation of testing methods.

11.3.2 Adapting the Statutory Framework

Taking the basic principles in Section 11.2 into account, and in view of the regulatory framework conditions for nanomaterials, the following principles have to be considered.

11.3.2.1 No General Moratorium for Engineered Nanomaterials in Food and Food Packaging

A general moratorium for any kind of engineered nanomaterial use in food and food packaging is currently not being favoured. Based on the available findings on market availability of food with engineered nanomaterials in Europe and their toxicological evaluations, there is currently no evidence for nanomaterials used in food products that have a major toxicological concern. The context is different for substances that are used outside Europe as dietary supplements e.g. nanoscale silver and gold that partly have a higher toxicological risk potential to humans,[2] and for which a positive effect on human diet has not been proven. Even though many of these supplements are not marketed in Europe, they are available to customers via the internet. In this case, however, only an EU or worldwide moratorium would be an effective instrument.

Another argument against a general moratorium is that possible chances associated with relatively safe applications of nanomaterials (e.g. in the area of packaging) would be undermined altogether. Nevertheless, a specific moratorium for the use of free nanoparticles, or for certain application types (e.g. nano-silver), could be the result of a societal dialogue (cf. Section 11.3.4).

11.3.2.2 No Issue of a 'Nanofood Law'

A 'nanofood law' also seems to be of no help. In fact, the existing regulatory instruments on food and food packaging should be adapted to nano-specific requirements. Nanomaterials are chemical substances, just like other materials or packaging substances, and may find their use in food products. As such, they should be subject to food legislation. It is, however, questionable whether there is a necessity for a special position through adaptation of the existing regulation on the basis of their chemical and/or physical behaviour. Each food or food additive producer or importer can find the corresponding rights and obligations within the existing regulatory framework, and is subject to them. If a new 'nanofood law' is developed, the complications of double regulation and cross-referencing can be foreseen. These could hamper the transparency of the existing regulatory framework for nanomaterials. Furthermore, there is the risk that producers and importers would question the applicability of the existing legislation for nanomaterials, and that they might be more restrictive with regard to development and adoption of voluntary measures (cf. Section 11.2).

In summary, a number of questions would have to be clarified for a nano-specific food law. This would include the definition of nanomaterials, the standardisation of measurement, testing and evaluation methods as well as the setting of limit values that currently do not exist or for which the basics are still under investigation in international standardisation bodies.

11.3.2.3 Possible Adaptation of Existing Food Provisions

Basically, the Novel Foods Regulation (EC) No 258/97 could be regarded as a 'nanofood law'. According to Art.1 (2) (f) of the Novel Food Regulation the

Regulation is applicable if 'foods and food ingredients to which has been applied a production process not currently used, where that process gives rise to significant changes in the composition or structure of the foods or food ingredients which affect their nutritional value, metabolism or level of undesirable substances.' The definition principally covers the use of nanotechnology in foods or food ingredients, underlined by the new Novel Foods Regulation currently under discussion and stating that nanotechnology is a 'new technology'.

However, the Novel Foods Regulation is not applicable to food additives, flavourings or extraction solvents nor to food packaging. Therefore, the existing laws relevant for food additives and food packaging as well as statutory instruments associated to these laws (discussed in Chapter 10) need to be adapted to the requirements of nanomaterials in food and food packaging, in consideration of the precautionary principle. In doing so, at first it should be clarified whether and how far food legislation in force on admissible food additives is valid for the corresponding nanoscale forms. This clarification can either take place within the legal provisions themselves or through administrative rulings. For that purpose it is necessary to standardise the definitions and nomenclature for nanomaterials as well as to promote the development of suitable testing procedures and monitoring methods.

Notification obligation. Whereas in the case of novel food, the food itself needs to be authorised, for all other food and food packaging applications only the additives are subject to authorisation. Furthermore, there is an uncertainty whether the use of nano form of an additive will require a specific authorisation when it is already approved as a macro-scale additives. In order to achieve transparency on the use of nanomaterials in food on the part of regulatory authorities, it is necessary to inform them accordingly. In that respect, it is recommended to introduce a notification obligation for the following cases.

- Producers and importers who put food or food packaging containing high-risk nanomaterials on the market should notify these to the responsible food authorities. In this case, a risk potential can to be assumed if scientific evidence exists on serious or irreversible harm or on a scientific plausible risk hypothesis for nanomaterials.
- Producers and importers who put food additives on the market that are already authorised and contain nanoscale fractions should – irrespective of a risk potential – also notify these to the responsible food authorities.

Due to the international dimension of the food and food packaging market, a European or worldwide provision for the notification obligation will be preferable to a national one for broader practical reasons.

Traceability. Traceability of food and substances in food is a core element for a producers' self-regulation. Producers should follow their obligation to

comply with legal requirements especially in respect to health protection to trace and follow a food, feed or substance intended to be, or expected to be incorporated into a food or feed, through all stages of production, processing and distribution. Traceability is a recognised basic principle of food legislation as well at international level (FAO/WHO Codex Alimentarius) and in the EU and has already been valid for certain products for many years. The traceability provides all actors the possibility to withdraw products with nanomaterials from the market after authorisation should they prove – on the basis of new scientific evidence – not to be safe. The legislator should thus check whether and how far the provisions for traceability are to be adopted to engineered nanomaterials and how the provisions can be applied in producers' practice.

Specific labelling. A labelling of nanomaterials used in the production process is recommended – especially with the purpose of tracing-back food products, for monitoring the presence of nanotechnology-derived ingredients and additives, and for giving consumers the freedom of choice. The labelling can be set up in line with existing labelling schemes for food and food additives, but should prescribe the full declaration of ingredients irrespective of the amount contained in the food. It is also recommended that the labelling codes are designed in such a way that nanoscale additives are identifiable, e.g. with the addition of an 'E' number including the size of the additive (e.g. E 551-N 50).

11.3.3 Corporate Responsibility

For manufacturers, processors and retailers of food and food packaging containing engineered nanomaterials, it is not sufficient to develop and distribute products that only meet legal standards. This has already occurred in other areas, e.g. with respect to pesticides or social aspects. Since the current legislation covers nanoproducts in principle, but needs to be adapted for the new nano-specific characteristics and risks, corporate responsibility in the development of nanotechnology products become much more important. A big responsibility, therefore, lies with the players in industry and business – particularly in such a transition period between the 'innovative advances' of developers compared to the 'succeeding' legislative framework. Thus, they are particularly obliged, through application of the precautionary principle, to precisely evaluate the effects of nanoproduct production and consumption with regard to health, environment and society. Hence, the key task is a consistent perception of product responsibility starting from development over to production, marketing and consumer information up to waste management.

This section will, therefore, introduce approaches that should be taken into account by manufacturers in the context of development and production of intrinsically safe substances and products. 'Intrinsically safe' means minimising the human and eco-toxicological risks over the whole product life cycle at the stage of product design.

Since risk minimisation always requires an integrated consideration of the probability and dimension of possible damages, such an approach always needs to consider human and environmental exposure as well as the toxicological risk potential associated with the utilised nanomaterials. Due to the currently incomplete state of knowledge with regard to the toxicological risk potential of nanomaterials, the following primary measures for risk minimisation have in the first instance the goal of reducing exposure of humans and the environment. Furthermore, they are also to reduce the (human and eco-) toxicological impact potential of the utilised nanomaterials.

Processing in closed facilities. Occupational exposure during normal operation can be avoided through closed operational management in nanomaterials' production and processing facilities. The existing facilities with open operational management can be upgraded accordingly with an encapsulation. However, it has to be taken into consideration that in case of processing in closed systems it is also necessary to clean and service the closed facilities regularly. Herewith, normally, a temporary opening of the facility is necessary which can in principle lead to human and environmental exposure to engineered nanomaterials.

Against this background, particular care is necessary to avoid disturbance of the closed facilities. In case a fully automated cleaning service is not possible, occupational health protection measures should be followed for the assigned staff. Moreover, a possible need to wipe up residues should be carried out strictly in wet conditions or with a liquid medium to avoid dust dispersion and thus exposure through inhalation.

The recommendation on processing in closed systems is also an important part of the already existing guidelines on occupational health and safety, e.g. 'Guidance for Handling and Use of Nanomaterials at the Workplace' issued by the German Chemical Industry Association (VCI) and the Federal Institute for Occupational Safety and Health (BAuA). Some manufacturers have also already developed guidelines on similar lines.[17]

Compatibility of production waste with existing waste management infrastructure. However, in addition to the interruptions of a closed processing during cleaning and servicing, it has to be taken into consideration that even in closed facilities waste and by-products are generated that need to be removed regularly.

In this respect, their subsequent management can lead to exposure of humans and especially the environment. In this context, dried nanomaterials are particularly problematic since they can cause dust emissions during decanting, transport and conditioning processes of waste management operations and thus have a negative impact on industrial safety.

Use of substances with a preferably low toxicological risk potential. In addition to the above-mentioned measures intended to minimise exposure during

the production process, a further risk management approach consists in the use of substances that are characterised through a particularly low toxicological risk potential. For food and food additives, corporate responsibility can be described in such a way that the toxicological safety of the used nanomaterials has to be guaranteed without doubt.

In view of the existing knowledge gaps, this means that the necessary intensification of human and eco-toxicological risk research is primarily a task for the producers and importers. Furthermore, they should respect the highest possible environmental and social standards; this can only be evaluated in relation to individual products.

According to the current state of knowledge, a comparably low toxicological risk potential is to be expected for nanomaterials especially if these either 'lose' their nano scale rapidly or if they are permanently bound in a matrix. Such a rapid 'loss' of the nano scale takes place, e.g. for nanomaterials that:

- have a high water or biological media solubility or
- can easily decompose into inoffensive substances through biochemical or physical processes.

In this context, it is recommended to check whether the utilised substances have a so-called 'GRAS certification'. GRAS stands for 'Generally Recognised as Safe' and is a denomination of the U.S. Federal Food and Drug Administration (FDA) for substances that have been shown to be harmless to health in tests or through long-term field experience. A classification under GRAS only takes place if there is consensus among the experts with regard to safety of use.

The list of substances with GRAS status is available on the internet at the FDA homepage. Normally, these substances are comparably near-natural chemicals that have a good solubility and/or are easily decomposable.[15]

For food packaging and food contact materials, a consistent perception of product responsibility means – for the developers in companies – to first of all identify the nanomaterial with the least toxicological risk potential. This is to be done in the context of a screening and selection process. Here, often a multitude of different nanomaterial options with the same functionality exist.[2]

Next to human-toxicological aspects, this selection process should also be led by (currently often still neglected) eco-toxicological impact potentials. In addition, for food packaging the application of a nanomaterial is to be preferred that contributes to minimising the exposure probability. For example, a direct contact of a nanomaterial used in packaging with the food should be avoided if migration from the packaging material cannot be excluded.

11.3.4 Scientific Assessment and Societal Dialogue During the Products' Development Process

In addition to the policy makers' and public authorities' important duty to establish necessary regulatory frameworks as a part of the overall risk

management, another major task is to initiate a societal dialogue on common goals of the sustainable application of nanomaterials in the food sector. The societal communication process should be organised in dialogues with all relevant actors. These include:

- research community
- manufacturers and processors
- retailers
- banks and insurance companies (especially reinsurances)
- NGOs
- consumers.

Taking into account the rather reserved communication policy of producers that can currently be observed, the first aim of a dialogue should be to develop trust and confidence. In the first instance, this is producers' responsibility. However, scientific experts as well as retailers can also make valuable contributions. Otherwise the danger exists that a debate similar to that on genetic engineering in food may be repeated. Transparent and credible information on nanotechnological products and production processes will contribute to consumers' confidence and to their freedom of choice. At the same time, the information needs of consumers with regard to real and individually perceived risks should be taken seriously.

Stakeholder involvement, however, needs to be based on a scientifically sound and interdisciplinary assessment of risks related to the use of nanomaterials in food and food packaging. Otherwise, the dialogue would focus rather on the individually perceived risks lacking a fact-oriented basis and most likely to mix risk assessment and risk evaluation. Thus, the risk assessment has to be carried out within an interdisciplinary scientific expert panel and prior to the societal communication process. In essence, this implies that the existing data gaps and uncertainties have to be tackled (cf. Section 11.3.1).

Within this context, a neutral, differentiated and appropriate characterisation of the different nanomaterials that are relevant for the food sector could be helpful. One important precondition for this is a registration obligation by the producers and marketers for these engineered nanomaterials as well as a 'safety roster' that contains information on market volume, on exposition and toxicological properties, on industrial safety as well as on environmental protection. In Switzerland, for example, the need for such a safety roster was expressed within the national action plan on engineered nanomaterials in 2008.[16]

The core aspect of the proposed public dialogue is the *evaluation* of risks, i.e. a common understanding on which nanomaterials are accepted by society for their use in the food sector and which nanomaterials are to be renounced for the time being due to knowledge gaps and due to an unacceptable benefit–risk relationship. It is stressed that any public dialogue needs to be based on case-by-case approach, in which different nanomaterials are assessed regarding their specific uses. Within this context, the benefit or the added value of the

nanoproducts has to be assessed and evaluated compared to the corresponding conventional equivalents. Again, it is the obligation of the policy makers and public authorities to involve all relevant stakeholders in this integrated risk and benefit evaluation process, *inter alia* in respect to achieve a consensus on societal value categories (cf. Section 11.2.3).

Furthermore, both the risk assessment of food and food packaging containing engineered nanomaterials and the dialogue process should not be designed as an add-on at the end of the development process of a new nanomaterial or a new application, but rather as an integral part of it. It is the clear advantage of risk assessment and risk evaluation at an early stage that in many cases alternative 'technological pathways' for a desired application exist, which can be evaluated promptly at the 'technological fork'. In this respect, one could eventually rely on the existing experiences and results from risk dialogues in other sectors.[15] Another important aspect that also needs to be discussed and considered in the context of a societal communication process is the question of risk communication and labelling of engineered nanomaterials in the food sector (in that respect cf. also the regulatory recommendations in Section 11.3.2). A public dialogue is considered to be the suitable forum for consumers and NGOs to name and substantiate their information needs with regard to labelling. Against this background, producers and distributors of food and food packaging containing engineered nanomaterials need to check the possibility to develop a nano quality information scheme along the supply chain. Elements of such a quality-based approach could be the following.

1. The substance or the preparation contains nanomaterials in terms of a definition that is generally recognised or sector-specific.
2. Under the application of the precautionary principle, the utilised nanomaterials have been subject to additional human and eco-toxicological tests compared to standard requirements under REACH; furthermore, in this case, the delivery of the standard record is to be demanded, regardless of whether the threshold of 1 t has been exceeded and that therefore elaboration of exposition scenarios for all intended applications of the nanomaterial is necessary.
3. Information gained in the context of these additional tests as well as their derived risk management measures allow for a safe use and will be communicated comprehensibly along the value chain through the safety data sheet.

In that way, the above outlined nano quality information scheme allows on the one hand having protection against misuse of the term 'nano' by non-serious producers and on the other hand having a security check of nanomaterials that is voluntary and goes beyond the current state of legislation. Furthermore, next to meeting consumers' information needs the claim for more transparency within the value chain is also recognised.

In order to ensure the credibility and the acceptance of such an information scheme, it should be awarded and controlled by an independent organisation

that is also generally accredited within the food sector. Beyond that, it would be welcomed if the information scheme was to be also supported by authorities. Eventually, all substances and preparations that have been awarded such a scheme should be listed in a publicly accessible database (e.g. on the internet) in order to facilitate the identification of relevant products by the users.

References

1. U. Eberle, D. Hayn, R. Rehaag and U. Simshäuser, (Eds.), *Ernährungswende. Eine Herausforderung für Politik, Unternehmen und Gesellschaft,* Ökom Verlag, München, 2006.
2. M. Möller, U. Eberle and A. Hermann, *Nanotechnologie im Bereich der Lebensmittel,* vdf Verlag, Zürich, 2009.
3. http://ec.europa.eu/consumers/safety/committees/ra_guidelines_workshop 11122007.pdf.
4. ad hoc Kommission, *Neuordnung der Verfahren und Strukturen zur Risikobewertung und Standardsetzung im gesundheitlichen Umweltschutz der Bundesrepublik Deutschland*, Abschlussbericht, June 2003.
5. U. Eberle, D. Hayn, R. Rehaag, U. Simshäuser, I. Stieß and F. Waskow, *Vorsorge im Handlungsfeld Umwelt-Ernährung-Gesundheit. Anforderungen an sozial-ökologische Vorsorge für eine Ernährungswende*, Discussion paper No. 12, August 2005, www.ernaehrungswende.de.
6. M. L. Socolof, *Life Cycle Assessment and Life Cycle Thinking*, Performance Track Teleseminar, www.epa.gov/perftrac/LCA%20teleseminar-1-26-05.ppt.
7. Environmental Management – Life Cycle Assessment – Principles and Framework (ISO 14040:2006).
8. Environmental Management – Life Cycle Assessment – Requirements and Guidelines (ISO 14044:2006).
9. R. Grießhammer, M. Buchert, C.-O. Gensch, K. Graulich, C. Hochfeld and I. Rüdenauer, *PROSA – Product Sustainability Assessment,* Guideline, Freiburg, 2007.
10. Rat von Sachverständigen für Umweltfragen (Hrsg.), Umwelt und Gesundheit, Risiken richtig einschätzen, Sondergutachten, 1999.
11. A. Grobe, O. Renn and A. Jaeger, *Risk Governance of Nanotechnology Applications in Food and Cosmetics,* a report for IRGC, Geneva, 2008.
12. Interessengemeinschaft Detailhandel Schweiz, Code of Conduct: Nanotechnologies, Version 5, February 2008, www.igdhs.ch/m/mandanten/190/download/CoC_Nanotechnologien_final_16_01_09_e.pdf.
13. C. Jones, Swiss food retailers demand information on nanotech, in Food Production Daily, 23 April 2008, www.foodproductiondaily.com/Processing/Swiss-food-retailers-demand-information-on-nanotech.
14. Health Council of the Netherlands, *Prudent Precaution*, The Hague, Health Council of the Netherlands, 2008, publication no. 2008/18E, p. 80.

15. *CONANO – Comparative Challenge of NANO Materials*, A Stakeholder Dialogue Project between Novartis International AG, Ciba Spezialitätenchemie AG, Öko-Institut e.V., Österreichisches Ökologie Institut, Stiftung Risiko-Dialog, Basel, Freiburg, Bregenz, St. Gallen, 2007, http://www.oeko.de/conano.
16. Eidgenössisches Departement des Innern EDI (eds.); Aktionsplan 'Synthetische Nanomaterialien', Bericht des Bundesrates vom 9, April 2008, Bern, 2008.
17. For example, 'Guide to safe manufacture and for activities involving nanoparticles at workplaces in BASF AG', http://www.basf.com/group/corporate/en/function/conversions:/publish/content/sustainability/dialogue/in-dialogue-with-politics/nanotechnology/images/BASF_Guide_to_safe_manufacture_and_for_activities_involving_nanoparticles.pdf.

CHAPTER 12

Knowns, Unknowns, and Unknown Unknowns

QASIM CHAUDHRY, RICHARD WATKINS AND LAURENCE CASTLE

The Food and Environment Research Agency, Sand Hutton, UK, York YO41 1LZ

12.1 Nanofood: Knowns and Unknowns

Every new technology brings a promise of new benefits. The uncertainties at the early stages of a technology may also raise certain concerns; such as over safety to human health and the environment, adequacy of risk management strategies, regulatory controls, and whether the benefits would be shared equitably within the society. Nanotechnologies are no exception to this. Whilst the debate over key issues and the research to remove uncertainties are both at early stages, the speed of industrial developments in this area has taken many by surprise. In particular, applications for a sensitive area like food are resurrecting many old issues and generating some new questions. Not surprisingly, there is also a degree of polarisation of opinions on the issues. On one hand are projections for the enormous benefits that the new technologies could bring, and on the other hand are the doomsday scenarios that associate every imaginable risk with nanotechnologies. The truth probably lies somewhere in between the two extreme views. The lack of clarity on some of the key issues – starting off with terms and definitions – has led to a situation of uncertainty, if not confusion, on the part of industry and the consumers alike. There also appears to be a trust deficit between some of the stakeholders. Large food corporations, some of

RSC Nanoscience & Nanotechnology No. 14
Nanotechnologies in Food
Edited by Qasim Chaudhry, Laurence Castle and Richard Watkins

Published by the Royal Society of Chemistry, www.rsc.org

whom had been actively engaged in nanotechnology research in recent years, do not want to be associated with the term 'nanotechnology' anymore due to the current sensitivities and uncertainties. Some NGOs have called for a moratorium, or an outright ban, on the use of nanotechnologies until they are proven to be safe to consumers and the environment. In consideration of such diverse points of view, this book has attempted to take a balanced view of the main issues in the light of the available knowledge. This chapter presents a summary of the different aspects and viewpoints in relation to application of nanotechnologies for food and related sectors. It is hoped that a holistic view of the issues will help clarify some of the uncertainties, so that at least some of the low-risk and the no-risk applications can be developed with confidence.

12.2 A Nano Matter of Definitions

The main nano-related features of ENMs (engineered nanomaterials) derive from their size, shape, specific surface area, surface chemistry, etc. A further important aspect is the potential for ENMs (especially free nanoparticles) to cross some biological membranes that act as barriers against entry of (larger) particulate materials. A number of definitions have been devised in recent years to capture the nano-related features of ENMs. Most notable of these include those proposed by RS/RAE (Royal Society and the Royal Academy of Engineering),[1] BSI (British Standards Institution),[2] ISO (International Organization for Standardization)[3] and SCENIHR (the EC's Scientific Committee on Emerging and Newly Identified Health Risks).[4] More recently, the European Food Safety Authority's Working Group has adopted definitions that are relevant to food and feed applications (EFSA, 2009).[5] A daunting task for encompassing nano-related features in a definition is to draw a boundary, on the basis of some physicochemical attribute(s) (most notably the size), beyond which properties of a material are likely to change significantly from the conventional bulk equivalents. Where a definition may have legal consequences, then legislators prefer a simple and clear-cut distinction, i.e. what is legislated or authorised and what is not. In this context, it is important to emphasise that the less than 100 nm size range used in the current definitions only loosely refers to the nanoscale at which a significant change in material properties is likely to take place. In reality, there is no clear size cut-off for the phenomenon, because the change in material properties in relation to size is a continuum. Although a significant change is more likely to happen below 100 nm size range, this does not mean it will not happen at sizes above 100 nm for some materials. The SCENIHR opinion on risk assessment of nanotechnologies[6] provides a number of relevant aspects to the definition of ENMs. One interesting suggestion is to place a minimum surface area of $60\,m^2\,g^{-1}$ or more for a material to be regarded a nanomaterial.

Another important aspect to consider is the physical form of ENMs as present in a real-life application. The properties of ENMs not only derive from physical dimensions (size/shape), but also from chemical composition and the high

surface energy and unbalanced surface forces. It is because of the surface forces that free nanoparticles tend to agglomerate or aggregate into larger structures. In agglomerated forms, the primary particles are held together by weak forces, such as van der Waals forces, whereas in aggregated forms they are bound strongly by covalent or metallic bonds. According to the ISO definition, the external surface area of agglomerates is similar to the sum of the surface areas of the individual components, whereas the external surface area of aggregates is significantly smaller than the sum of the calculated surface areas of the individual components. In short, agglomerates can still offer nano-related properties because of the primary particle surface areas, albeit with a diminished ability to penetrate biological barriers. Agglomerates can also shed primary nanoparticulate fragments, for example, in the presence of surfactants, or by action of other (bio)-chemical agents. Compared to this, aggregates will not release nanoparticles under normal circumstances, and they behave more like conventional larger bulk materials than nanomaterials, albeit with increased surface areas.

It emerges from overview of the applications of ENMs in food, and the possible hazards that they might pose, that a clear differentiation needs to be drawn between those that are solubilised or digested in the GI tract (also termed as 'soft' nanomaterials), and those that remain insoluble and undigested, and may be bio-persistent (termed as 'hard' nanomaterials). Once solubilised or assimilated, the ENMs lose their nano features, and thereafter are not likely to behave any differently from the bulk equivalents. A toxicological hazard may still be associated with 'soft' ENM types, but it is likely to derive from chemical constituents rather than the nano features. With a few exceptions, the vast majority of the naturally occurring or processed food nanostructures, such as lipid micelles and emulsions, will fall into this category. The possible exceptions could be the nano-delivery systems that have been deliberately designed to cross the GI tract intact, taking the encapsulated substances into the circulatory system. Some processed food nanostructures may also prove to be different from those that exist in foods naturally, and therefore may be dealt with in the body differently. However, this area has been discussed at length in Chapter 4 from the perspective of molecular structures in complex multi-component foods. It is clear that many, if not most, existing foods sold today already have (soft) food nanostructures present. These either occur naturally or are formed or modified as part of the food processing technologies used conventionally. Nanotechnologies and nanosciences therefore also provide new analytical and modelling tools for visualising, understanding and then controlling the structure–function relationships between different food components. These tools and the understanding may be used for rational selection, processing, or design of food materials in order to improve food quality including nutritional aspects as well as aesthetics such as taste, texture, aroma, etc. On the whole, this area of application should not raise any special safety concerns. This being so, it seems likely that any evaluation of these foods with so-called 'soft' nanostructures would focus mainly on the questions of digestibility and bioavailability compared to the conventional food counterparts. In fact, such applications need not be branded 'nanotechnology' *per se*, as the

safety issues in relation to ENMs for food applications relate mainly to the use of 'hard' ENMs.

12.3 New for Old?

The proliferation of nanotechnologies into certain sensitive consumer sectors such as food has, nevertheless, raised a number of questions, the fundamental of which is whether there is a need for such applications. Chapters 1, 5 and 6 have discussed the known and projected applications of nanotechnologies for food and related sectors. These can be judged on a number of merits – for example, whether they have been developed in response to a real need, to address a market gap, or whether a 'need' has been invented *post facto* to justify new applications for a technology originally designed for some other purpose. It could be envisaged from the cross-cutting and enabling nature of nanotechnologies that they would not remain limited to any one sector, and it was inevitable that the concepts and materials developed for one sector would gradually find way into other sectors. At the same time, it needs a critical look to see whether and how the new materials and applications are any different, or indeed superior, compared to those already used by the industry. A way to gain insight into these aspects is to analyse the benefits that nanotechnologies could offer at best, and the risks they might pose at worst. A cross-sector comparison of risks and benefits in this case is difficult because of the broad-ranging, multi-sector applications of nanotechnologies, where the use of a particular ENM for one application (e.g. for food packaging) may be more acceptable, e.g. from health and safety points of view, than another (e.g. for food). The merits of each application, therefore, can be considered on a case-by-case basis. It is also important for a new emergent field like nanotechnologies to know who is likely to benefit most from the new developments, and how the developments are likely to be seen by the consumer and the regulatory authorities.

The overview of the relevant products and applications presented in this book indicates that at least some of the materials and concepts have been derived from research in other sectors, particularly the medicine and cosmetics sectors.[7,8] The R&D into nano-delivery systems for targeted drug delivery, and nano-sized ingredients for cosmetics far precede the comparable applications for food. Many themes, nevertheless, seem to be common between the three sectors, as they are based on similar principles, e.g. novel functionalities and enhanced uptake and bioavailability of ENMs. For food applications, there are other additional drivers for nanotechnology applications, such as improvements in taste, stability and texture of foodstuffs, etc. This is evident from the first array of products in that they mainly fall into the areas where boundaries between the food, medicine and cosmetic sectors are not so clear. Thus a large majority of the currently available products belongs to (health)food supplements, nutraceuticals and cosmeceuticals, etc. Another reason for this may, however, be that, like any new technology, the first set of applications is usually for high value products. This, nevertheless, implies that

the current and short-term predicted applications for the mainstream food and beverage products are only marginal. Despite this, considering the size and breadth of the global food sector (worth between 3 and 4 trillion US$ per annum), and the potential versatility of nanotechnology applications, even a modest level of market penetration has the potential to attain a sizeable market value within a short period of time.[7,9]

12.4 A Nano Vision for the Future Food

The broad-ranging and emergent nature of nanotechnologies makes it difficult to predict how applications in the food area will evolve over the long-term. The current and short-term projected applications are certainly multi-faceted. As discussed in Chapter 1, the main drivers for such applications centre on new properties and compositions of food ingredients and additives, and novel functionalities of packaging materials. The likely convergence of nanotechnologies with other enabling technologies, in particular information technology, biotechnology and cognitive sciences, is expected to pave the way for many more new and potentially yet-unforeseen developments. A current example is that of nano(bio)sensors which, although still evolving, are already finding applications in 'Smart' packaging concepts, and have the potential for large-scale adoption in the wider food production chain. At present, however, it is only possible to draw a hazy vision of the future shape and impact of nanotechnologies on food and related sectors due to the vast array of known and potential applications, and the uncertainties of consumer acceptance and uptake.

12.4.1 A Beneficial Technology?

One of the obvious benefits of substituting conventional bulk equivalents with nano-sized materials can be a reduction in the use of synthetic ingredients and additives in food. The notion 'less is more' certainly seems to fit in well with the use of a nano form of food ingredients and additives. Due to the larger surface areas, a relatively small amount of an ENM may provide a level of functionality that would require a greater amount of the bulk equivalent. Other benefits may derive from the ability of nano-sized water-insoluble additives to disperse uniformly in foodstuffs, allowing a reduction in the use of fat or surfactants in food and beverage products. The nano formulations of supplements may also offer a greater uptake, absorption and bioavailability in the body compared to bulk equivalents.

From the overview of developments in the food area presented in Chapter 5, a major thrust of the current applications seem to be on the development of processed nanostructures in foodstuffs. For this, some innovative approaches have been used to develop stable nanomicelles and liposomes. As the technology matures, such applications are likely to offer novel or improved food tastes, textures and mouth sensations. Examples are low-fat foods, such as a

cream or spread, which tastes like the full-fat version. Subject to consumer acceptance, and the level of market penetration, such foods could make a significant contribution towards addressing the growing problem of obesity by enabling a reduction in the dietary intake of fat, whilst still offering the consumer tasteful food products. The target market for such applications could be premium brands of healthy option foods. The main initial focus of such applications is likely to be for dairy products, and other conventionally high-fat food items such as sauces and dressings.

A significant impact of nanotechnologies is likely to come from the use of nano-sized additives. A comparatively smaller amount of a nano-sized additive could suffice to deliver the same perception of a taste or a flavour. This is anticipated to enable a reduction in the amount of a range of food additives, such as colours, flavouring agents, preservatives, etc., whilst offering similar or improved aesthetic, nutritional and health benefits. If such expectations prove to be a reality, this would open up new opportunities for a significant reduction in the amount of many ingredients and additives, such as salt, sugar, fat, preservatives, spices and other culinary agents. Subject to such nano-additives not posing a health risk, their use could certainly benefit the consumer as the foods would contain lesser amounts of a variety of additives. This would also benefit the industry as they would be able to introduce more innovative products on the market with new or enhanced tastes, flavours and mouth-sensations, whilst cutting down on artificial additives. The target market for such applications could be envisaged for a wide range of food and beverage products.

The emerging methodology of nano-encapsulation is likely to find many applications in developing food additives that are not only able to withstand harsh food processing conditions, but also release the encapsulated substances when required. The technology could be used for numerous versatile applications. For example, a nano-encapsulated nutrient, supplement or flavour could offer a greater impact if it was released in the mouth. The technology would also enable masking some undesirable tastes and flavours. This could open the door for some health-promoting supplements in foodstuffs, the use of which is currently problematic due to unsavoury taste or flavour. Examples include plant-derived polyphenolics, garlic extract, fish oils, *etc*. The use of nano-encapsulation could also enable development of products based on some novel and innovative ideas, such as those that would change colour or flavour, or release nutrients and supplements, at consumer's choice.[7]

The use of micelle- or liposome-based nanodelivery systems in food may offer another substantial benefit to the consumer in terms of enhanced uptake, absorption and bioavailability of the nano-formulated dietary supplements compared to conventional bulk equivalents. This is likely to work well for some supplements that are not easily absorbed in the body, such as certain antioxidants, and minerals like magnesium. A better uptake and bioavailability of supplements, such as vitamins and antioxidants, could benefit consumers in general, and certain age groups in particular. This might even enable tailoring of foods for different age-groups and lifestyles.[7] Potential benefits like these are

already attracting a lot of commercial interest in the use of nano-formulated supplements and bioactives for a variety of (health)food applications. Similar approaches are also understood to be under R&D for animal feed applications.

As discussed in Chapter 6, nanotechnology derived packaging materials offer an opportunity for lightweight but stronger food packaging that can keep food secure during transportation, fresh for longer during storage and safe from microbial pathogens. Any extension in shelf-life of food products should contribute towards reducing the waste of foodstuffs. Antibacterial nanocoatings on food preparation surfaces could help maintain hygiene during food processing, such as meat cutting machinery in abattoirs, and food preparation/processing surfaces and conveyer belts.

The use of 'Smart' labels that incorporate ENMs should make food products easy to track down in the supply chain. Such labels could also make a significant contribution to food safety in terms of visual warnings to the consumer if a packaged food product was subjected to a freeze–thaw–refreeze process in the supply chain, the integrity of a product packaged under vacuum or inert atmosphere was compromised, a product had passed the sell-by date, or the food inside a package had started to deteriorate. These intelligent labels will therefore not only help safeguard consumer health, but should also contribute towards reducing the undue wastage of foodstuffs.

12.4.2 A Risky Technology?

Whilst nanotechnologies offer numerous opportunities for innovation in a wide range of sectors, certain applications have also raised concerns in relation to consumer safety, ethical, policy and regulatory aspects. The biggest challenges currently facing the industry are how to identify and exploit the potential benefits of the new technology without undue risk to the consumer and the environment, and how to communicate the benefits and risks effectively. It is of note that the term 'risk' reflects both hazard and exposure, where the absence of one will essentially mean no risk. Thus whilst it is important to consider the nature of a hazard that might be associated with the use of an ENM; in terms of risk, it must be considered in conjunction with the likelihood, the extent and the frequency of exposure.

A number of recent reports have assessed whether the current risk assessment paradigm, designed for conventional substances, would be applicable to ENMs. The paradigm involves identification and characterisation of hazard through a well-defined series of tests aimed at determining the physicochemical properties and (eco)toxicological effects, and assessing the exposure through identification of the route, the likelihood and the extent of exposure. It is generally concluded that the existing risk assessment paradigm would, in principle, be also applicable to ENMs. However, it has also been pointed out that the current testing methodologies need adaptation in view of the special features of ENMs, e.g. insoluble particulate nature, possible agglomeration, binding of other moieties on particle surface, *etc.*[6,10,11]

12.4.2.1 Potential Health Hazards

As mentioned before, an ENM will have a certain chemical composition in addition to the nano-related size/shape features. The behaviour, interaction, fate and effects of an ENM will inevitably be influenced both by the nano features and the nature of the chemical(s) that make up the ENM. Like other chemical substances, some ENMs may pose a hazard to human health and the environment due to their inherent chemical nature, and also that such effects/impacts may be exacerbated because of the nano features, such as in terms of a greater delivery of the chemicals to a target site, or to a new target site. There is an enormous amount of experimental data on biological effects of micro- and macro-sized particulate materials, but extrapolation from these data to gain a meaningful insight into the likely effects of ENMs may be difficult.

Chapter 8 has discussed different (potential) health hazards that may arise from exposure to ENMs. It is known that ENMs can exhibit different physicochemical properties from conventional bulk equivalents. A shift in properties may also lead to a deviation in biological effects. It is clear from the currently available knowledge presented in Chapters 8, 9 and 11 that the main concerns over potential health risks relate to exposure to free nanoparticles, especially those that are insoluble, and which can remain indigestible and bio-persistent in the body (i.e. 'hard' nanoparticles). It is also worth noting that a vast majority of the existing scientific evidence in relation to ENM hazards relates either to exposure via the inhalation route, or to *in vitro* assays. There is, nevertheless, emerging evidence from both *in vitro* assays and the few *in vivo* studies that free nanoparticles are able to get through different biological barriers,[12,13] and thus may potentially reach new targets in the body that would otherwise be protected against entry of larger particulate materials. Thus, at least on theoretical grounds, the target organs for ENMs may be different from those expected from conventional forms of the same compound. Once inside target organ, the large (and potentially reactive) surfaces of the nanoparticulates may interact with different biological moieties and may interfere with some cellular mechanisms. Such interactions may lead to harmful health effects. For example, exposure to some ENMs has been shown to cause increased production of oxyradicals and potential oxidative damage to the cell.[14,15] The good news is that those ENMs that are composed of toxic chemicals are unlikely to be intentionally used in food applications. Thus, the 'priority' ENMs of concern for such applications will be those that are in the form of insoluble free nanoparticles or agglomerates, and which are likely to remain in the body undigested and bio-persistent following ingestion via food and drink. The dietary intake of some metallic ENMs that have strong antimicrobial activity could perturb the gut natural microflora. Similarly, an enhanced absorption and bioavailability of some nano-additives may give rise to a greater internal exposure, with higher plasma concentrations or higher area-under-the-curve exposure. In the case of some food additives, such as artificial colours, flavouring agents, preservatives, etc., the increased bioavailability may require a reassessment of the acceptable daily intake (ADI) value.

As mentioned before, the indication of hazard alone does not mean a risk. In a risk assessment perspective, a hazard needs to be seen in conjunction with the likely exposure. It is, therefore, important to stress that the full nature of the risk from the ingestion of different ENMs via food and drinks is currently not known. As the toxicological hazard of an ENM may be different from equivalent bulk material, the toxicological profiling will need to focus on long-term studies that are followed up by histopathological investigations to establish the expected and unexpected target site(s). As any epidemiological evidence on potential hazard is not likely to emerge in the immediate future, there is at least a theoretical possibility that the use of some 'hard' ENMs in food applications may pose a yet uncharacterised health hazard. There is, therefore, a need for assessment of the potential risks on a case-by-case basis.[5]

12.4.2.2 Likelihood of Exposure

An essential element of chemical risk assessment is the information on the route, the likelihood, the extent and the frequency of exposure to a hazardous substance. As mentioned before, the current level of ENM application for food and drinks is at an elementary level. Any significant consumer exposure to dietary ENMs at present is, therefore, unlikely. However, as more products and applications are expected to emerge with time, the likelihood and extent of exposure may become significant in the future. In this regard, a number of knowledge gaps will need addressing to enable exposure assessment. An important need in this regard would be the information on the likely behaviour, interaction and fate of ENMs in food and in the gastrointestinal (GI) tract, and absorption and bioavailability in the body. As ENMs (especially free nanoparticles) have high surface free energies,[16] they will tend to agglomerate or bind other moieties on their surfaces. Therefore, most ENMs in food will be unlikely to remain in a free particulate form in the food or in the GI tract, and will undergo different transformations; such as agglomeration, binding with food components, reaction with stomach acid, breakdown by digestive enzymes, etc. ENMs are also known to become coated with different (bio)-molecules, especially proteins.[17] Some of the moieties bound to the surface may even direct the ENMs to specific locations in the body.[17] For assessment of the potential risk, it will also be important to consider any major shift in the ADME (absorption, distribution, metabolism, excretion) properties, and the likely bio-persistence of ENMs in comparison with the conventional bulk equivalents. This will require the generation of new knowledge in respect to some of the aspects. It thus appears unlikely that all the required elements for risk assessment will be available in the immediate future. In view of this, the risk assessors and regulators will need to consider wider safety margins, especially to applications that involve the use of 'hard' nanoparticles or their agglomerates.

Another route of potential exposure is from consumption of animal products. ENMs are under R&D for animal feed applications. The animal feed industry is

one of the most innovative sectors. It is driven by narrow profit margins for commodity products towards innovations giving greater animal productivity. It will be necessary to investigate if ENMs used in animal feed could enter the human food chain in milk, eggs, meats and other animal products. This will need ADME studies in the same way as foreseen earlier to investigate the fate of ENMs if any human exposure was to occur from use in foods.

12.4.3 Likely Beneficiaries and Vulnerables

It is clear from the overview of relevant applications of nanotechnologies, presented in Chapters 5 and 6, and the anticipated benefits discussed in Chapter 7 and Section 12.4.1, that it will be the consumer who is likely to benefit most from the new developments in one way or another. The industry will inevitably benefit too from the applications of the new technology, but the main thrust of the current and projected developments seems to be directed towards addressing the long-term needs of the consumer. For example, they will offer tasteful food products, and yet with lesser amounts of fat, salt, artificial colours, flavours and preservatives. The stealth way of using health-promoting food ingredients in nano-encapsulated form will further help maintain healthy lifestyles through the consumption of everyday foodstuffs. These developments could make a major contribution towards reducing the incidence of a number of food-related illnesses – such as obesity, diabetes, high blood pressure, etc. Similarly, the maintenance of hygiene during food production by nanotechnologies will contribute towards reducing food-borne diseases, again mainly for the benefit of the consumer. Increased shelf-life of food products will certainly reduce the amount of food wasted. According to the United Nations, the world population is set to reach 9 billion by 2050, and this will require a 70 per cent rise in global food production to avert major shortages. With global markets setting commodities prices globally, reducing the amount of food wasted in all countries has a clear part to play in making food available to all. The enhancement in absorption and bioavailability of dietary supplements will help support a healthy lifestyle for all, but especially the vulnerable age groups.

Unfortunately, the consumers will also be the most vulnerable party if the use of ENMs in food proves to pose (a yet-unidentified) health risk. The intimate nature of the exposure via consumption of food, the likely frequency of consumption through everyday food products, and the potential scale of population exposure would mean that there might be many more individuals exposed compared to any other sector application of nanotechnologies. However, food laws in most countries provide a reassurance against such a happening (Chapter 10 and Section 12.4.6).

12.4.4 Consumer Attitudes

Experts are often baffled by the failure of consumers to respond to their expert advice. Consumers continue to eat too much of foods containing high levels of

calories, salt, saturated fats, etc. whilst rejecting potentially healthy foods if made by production processes perceived as high-tech and 'unnatural'.

A welcome synopsis presented in Chapter 2 discusses the psychology behind our choice of foods, and provides an explanation as to why our reactions to rationally presented evidence sometimes appears so contrary. It appears that our sensitivity to the use of new technologies in producing our food is particularly heightened, and despite the intuitive appeal of all the benefits offered by nanotechnology, the new technology will not receive our automatic acceptance. The chapter also discusses the challenges for the food processing sector and the potential approaches that could be used to assess and inform attitudes. It is evident that effective research and development strategies for this new technology will need to encompass the consumer psychology if this technology is to fulfil its potential. Public engagement and informed dialogue are the key if we are to avoid the furore that has dogged the introduction of other new technologies in recent years such as food irradiation or genetic modification (GM).

Chapter 3 assesses how public attitudes to new food technologies and the key 'actors' in the debate (scientists, consumer groups, industry, the media and environmental groups) have changed over recent years and how these changes may impact on our acceptance of nanotechnologies in food. For a new technology, like nanotechnologies, the public must provide, in effect, a 'licence to produce' before the new products can come to market. The current level of public knowledge of the risks and benefits of nanotechnology is very low. One of the hard-learned lessons from the GM debate is that the vast majority of European people are not really interested in science unless they have a personal need. Their interest in the science and technology debate is largely a matter of spectacle, entertainment or controversy. Therefore informed engagement on nanofoods will be a challenge. The fundamental questions in people's minds are 'Whose agenda is it?', 'Whose interests are being served?' It is now becoming clear that knowledge and understanding of the science and technology is relatively unimportant in forming public perception and public opinion. The good news from the surveys of public opinion is that consumers perceive nanotechnologies in a positive light. While still unfamiliar to many, more were optimistic about nanotechnology in 2005 than in 2002, the ratio of optimists to pessimists being eight to one. Nanotechnology is perceived as useful to society, morally acceptable and not risky. Another interesting aspect that has appeared from the surveys is that, in comparison to people in the USA and Canada, Europeans see nanotechnologies as more useful and have a greater confidence in regulation.

Chapter 3 also presents possible ways to move the nano(bio)technology debate forward including the need to encourage and support scientists to explain their science and its significance for the general good. A major challenge in this regard is to avoid some of the projected benefits of nanofoods being seen by the consumers to mainly benefit the food industry. An obvious question in this regard is whether nanofood products offer any strikingly added benefit (health, nutritional, cost or other) to a consumer compared to a comparable non-nanoproduct. The answer may not be a straightforward one, as each

application is likely to be viewed differently by different stakeholders, depending on how the risks and benefits are 'communicated' and/or perceived. For example, if a nanotechnology derived packaging is used to increase the shelf-life of a food product, it may be seen either way by a consumer. If it has been used to extend the sell-by date of a food product, it will certainly be seen as mainly benefiting the industry. Although an extended sell-by date will cut down on food waste, the consumer will receive an older product – although no less fresh by some definitions – at the end of the extended shelf-life. A reconciling solution may be that where the new technology is used to extend shelf-life, the existing sell-by date is maintained. This will certainly offer a benefit to the consumer in terms of a higher-quality product at the end of the sell-by date. In the same way, a nanotechnology-derived antimicrobial packaging may be seen by the consumer as a camouflage for poor hygiene during food processing/storage. However, an active packaging that releases preservatives only when triggered by rough handling or transport abuse, or when microbial activity initiates in the packaged food, but is otherwise free of preservatives, is likely to be seen more favourably. Nanocoatings are also likely to be more acceptable to consumers, especially when they are on the outside of food packaging rather than on the inside. Smart labels incorporating nanosensors will certainly help assuring product quality and safety. Examples are identifying if a package has been tampered with or if food has started to deteriorate due to microbial spoilage. However, the current high cost of such labels and possible issues in implementing them into the existing systems may be a prohibitive factor for large-scale uptake by the industry. In the short-term, therefore, the use of 'smart' labels is likely to be limited to high-value products. The incorporation of radio frequency identification display (RFID) technology into nanosensors will add a very useful dimension in terms of traceability of food products during transit or in the event of a product recall. However, their use is also likely to evoke a controversy over consumer privacy issues.

12.4.5 Unknown Unknowns

The overview of different aspects of nanotechnology applications presented in this book indicates a number of major knowledge gaps in relation to both properties and effects of ENMs. The unavailability of robust methodologies for detection and characterisation of ENMs at low concentrations in food and drinks has left a virtual total lack of knowledge in regard to the behaviour and fate of ENMs in food and drinks. A number of factors are likely to influence the ADME properties of ENMs. The most obvious of these include size, shape, surface charge, surface chemistry, functional groups, solubility, degree of agglomeration/aggregation, chemical reactivity, etc., but there are likely to be other yet-unknown physicochemical features. The influence of such variables on the toxicological dose–response relationships of different ENMs has not yet been characterised. It is thus not clear whether a nano-additive will bind to other food components, agglomerate or remain available in the form of free

particles in the GI tract. The potential accumulation and bio-persistence of ENMs in different organs is also largely unknown, as are the potential additive effects of different ENMs acting together by a common mode of action that may need a combined estimate of exposure.

It is known that insoluble ENMs can adsorb, adsorb or bind different compounds and moieties on surfaces,[16] including proteins.[17] For example, iron oxide (magnetite) nanoparticles adsorb and transport different toxic elements, including arsenic in water.[18] A major unknown in this regard is whether some ENMs would transport other compounds adsorbed/bound to their surfaces into cells. At present, there is no direct evidence for this 'Trojan Horse' effect, but there are some possible indirect indications. For example, immunisation with carbon soot (particle size ~ 500 nm) has been reported to yield specific antibodies to polyaromatic hydrocarbons (PAHs),[19] indicating that the particles were probably acting as a carrier of PAHs. Another study[20] showed enhanced toxicity of phenanthrene in the presence of C_{60} fullerene aggregates (~ 200 nm) to algae and daphnids. The increase in toxicity was judged to be due to the delivery of sorbed phenanthrene to cell membranes, which is the site of toxic action of phenanthrene.

The risk assessment of ENMs requires an understanding of the dose–effect relationship. It is currently uncertain how best the dose of an ENM can be expressed. It appears that the use of conventional weight metric alone may not be appropriate for ENMs, and a combination of other measures, such as surface area, shape, surface charge, particle number, etc. may also be needed.[21] The available information on the underlying mechanisms for the ENM toxicity is also very sparse. Although the induction of reactive oxygen species, oxidative stress, inflammatory responses and apoptosis all point to a possible role of mitochondrial function,[21] there might be other mechanistic routes to ENM toxicity. For example, the presence of insoluble catalytic surfaces of some ENMs inside cells may impair some other yet-uncharacterised cellular functions. Appropriately adapted toxicological testing methodologies should uncover the 'anticipated' effects, but the 'unknown unknown' in this regard are whether some ENMs will exhibit unexpected toxicological effects, and whether the current testing methodologies would be able to detect them. In view of these uncertainties, it is imperative that toxicological profiling research focuses on experiments that use repeated doses over prolonged periods, and are followed up by histopathological investigations to identify any new unexpected targets for ENMs. The use of toxicogenomics and metabolomics approaches could also be very helpful in profiling any yet-unknown effects of ENMs.

12.4.6 Regulation Soft or Hard?

A few studies have assessed the relevance and adequacy of existing regulatory frameworks to identify any potential inadequacies and gaps in relation to the anticipated risks from applications of nanotechnologies for food.[22–25] A review of the regulatory aspects, presented in Chapter 10, indicates that in many cases

pre-market evaluation procedures for food products exist that should be applicable to nanotechnology applications. Examples of these include horizontal legislation, such as the general food laws and chemical safety laws, and vertical regulations, such as those relating to food additives, novel foods, specific health claims, food contact materials, water quality and other specific regulations relating to the use of certain chemicals (such as biocides, pesticides, veterinary medicines, etc.). Other environmental regulations may also capture the unintentional or accidental presence of ENMs in agri-food products. In Europe, the use of the precautionary principle provides an additional safeguard against potential health hazards from the use of ENMs in food. Recourse to the precautionary principle would be in situations where a potent harmful effect deriving from a product or process has been identified, but the existing scientific evaluation does not allow the risk to be determined with sufficient certainty.

Unless based on an exclusive case-by-case pre-market approval system, an effective regulatory framework will, however, need to provide a set of clear definitions that encompasses the distinctive properties of nano-ingredients and additives, a clearly defined responsibility and liability for relevant products and applications, and appropriate permissible limits that relate to the (potential) effects of ENMs in food. The findings of the review presented in Chapter 10 conclude from a number of recent reports that the current regulatory frameworks for food and food contact materials (FCMs) within a number of jurisdictions, such as the EU, the USA and Australia, are broad enough to capture foods and FCMs derived from nanotechnologies. These frameworks may not have been designed specifically to cope with some of the new challenges posed by nanotechnologies, but there are proposals to recast some of the relevant regulatory instruments, such as the European Regulation 258/97 (the Novel Foods Regulation), to remove any uncertainties in regard to the use of nanotechnologies for food. Considering the global nature of the business in the food and related sectors, any amendments to the existing regulatory instruments will need to take into account other international frameworks in order to develop a harmonised strategy for the governance of nanotechnology risks.

12.5 A Way Forward

The developments in the application of nanotechnologies for consumer products have led to a number of concerns over consumer and environmental safety. A close scrutiny of the concerns, however, provides more clarity over the issues. For example, it is clear from the current state-of-knowledge that the applications for food and related sectors are more likely to bring gradual but ultimately far-reaching benefits to the consumer and the industry, rather than some major step-change applications. It is also clear that many of the projected risks are more ‘hypothetical’ than supported by the current scientific evidence. In this respect, they presently have the character of concerns rather than risks. This is not to say that some yet-unforeseen hazards and risks may not come to surface in the future. But that is true for the risk assessment and risk

management of all products and processes. Some reassurance comes from the fact that there are tough regulatory controls in place to mitigate such risks. A further reassuring aspect is the food industry's overall approach to application of nanotechnologies, which has been very pragmatic so far. Unlike some other sectors, the food industry has not pushed for any nano-enabled products unnecessarily, or just for the sake of short-term commercial gains. Whilst this is true for large firms, who are also likely to carry out all the necessary safety checks before placing a nanofood product on the market, some smaller firms in certain parts of the world may be less proactive in this regard. However, safeguarding against such activities should not necessarily need a legislative solution, and they may be controlled through industry's own self-regulatory measures. The fact that the industry will ultimately be liable for the safety of marketed nanofood products necessitates a proactive and transparent approach on the part of the industry to ensure safe use of the technology in their products. For example, through establishment of a watchdog, the industry can ensure that only safe nanotechnologies have been used, a strict quality control of materials and products has been maintained and relevant regulations have been complied with.

The discussion presented in this book makes it clear that a number of applications will probably carry either a low- or no-risk compared to others. One example is the use of conventional food processing technologies in a directed way to form nanostructures in foodstuffs such as enhanced emulsions and modified starches. This probably should not raise any special concerns since the techniques have been used by the industry for decades, but in a non-directed way on an empirical basis. A second example is the use of nanomaterials in food contact plastics such as food packaging. In this case, the nanomaterials should be firmly embedded and anchored within the plastic with effectively zero potential for migration into the food.[26] In the absence of exposure, the risk to consumers would be insignificant. So what of other applications that are not so clear-cut. A way forward is to classify ENMs in food products that allows their assessment, regulation and labelling based on the likely persistence and accumulation of non-metabolisable ENMs in the body. Such a procedure can be developed on the basis of a conceptual risk assessment, in the light of available knowledge. This will allow some of the low- or no-risk applications to move ahead with confidence, whilst other more contentious ones can await detailed case-by-case safety evaluations, and/or approval where necessary. The key to the success of the technology, however, lies in the 'licence to produce' from the public that can only be obtained through building confidence, trust and acceptance. In this respect, the industry could consider voluntary selective use of labelling for nano-enabled products, especially those that contain 'hard' non-metabolisable, bio-persistent ENMs.

The potential impacts of nanotechnologies to improve the way our food is produced, processed, packaged, transported, stored and consumed has already attracted the attention of a number of research groups. In due course, research in this area is likely to provide answers to the issues and concerns highlighted in this book. The safety aspects of future nanofoods are also being carefully

scrutinised by a number of expert groups both at national and international levels. The outcome of these activities will inform policymakers to take steps to ensure that the new technologies enter the food arena in a manner that is both beneficial and safe for the consumer and the environment.

References

1. Royal Society and Royal Academy of Engineering, UK, *Nanoscience and Nanotechnologies: Opportunities and Uncertainties*, July 2004, www.nanotec.org.uk/finalReport.htm.
2. BSI (British Standards Institution), Publicly Available Specifications, PAS 131, PAS 132, PAS 133, PAS 134, PAS 135, PAS 136, www.bsi-global.com.
3. ISO, Nanotechnologies – Terminology and definitions for nano-objects – Nanoparticle, nanofibre and nanoplate, 2008, Reference number ISO/TS 27687:2008(E).
4. SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks), *Opinion on the Scientific Aspects of the Existing and Proposed Definitions Relating to Products of Nanoscience and Nanotechnologies*, 29 November 2007, http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_012.pdf.
5. European Food Safety Authority, *The EFSA J.*, 2009, **958**, 1–39.
6. SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks), *Risk Assessment of Products of Nanotechnologies*, 19 January 2009.
7. Cientifica Report, *Nanotechnologies in the Food Industry*, 2006.
8. Q. Chaudhry, M. Scotter, J. Blackburn, B. Ross, A. Boxall, L. Castle, R. Aitken and R. Watkins, *Food Addit. Contam.*, 2008, **25**, 241–258.
9. Helmut Kaiser Consultancy, *Study: Nanotechnology in Food and Food Processing Industry Worldwide*, 2003–2006–2010–2015, 2004, www.hkc22.com/Nanofood.html.
10. S. Rocks, S. Pollard, R. Dorey, L. Levy, P. Harrison and R. Handy, *Comparison of RA Approaches for Manufactured Nanomaterials*, 2008, Defra, London.
11. OECD Working Party on Manufactured Nanomaterials Preliminary, Review of OECD Test Guidelines for their Applicability to Manufactured Nanomaterials, ENV/CHEM/NANO(2009)6/REV1, Environment Directorate Organisation For Economic Co-operation and Development, Paris, 2009.
12. M. A. Chen and A. von Mikecz, *Exp. Cell Res.*, 2005, **305**, 51–62.
13. M. Geiser, B. Rothen-Rutishauser, N. Kapp, S. Schürch, W. Kreyling, H. Schulz, M. Semmler, V. Im Hof, J. Heyder and P. Gehr, *Environ. Health Perspec.*, 2005, **113**, 1555–1560.
14. E. Oberdorster, *Environ. Health Perspec.*, 2004, **112**, 1058–1062.
15. N. Li, C. Sioutas, A. Cho, D. Schmitz, C. Misra, J. Sempf, M. Wang, T. Oberley, J. Froines and A. Nel, *Environ. Health Perspec.*, 2003, **111**, 455–460.

16. P. Šimon and E. Joner, *J. Food Nutr. Res.*, 2008, **47**, 51–59.
17. I. Lynch and K. A. Dawson, *Nano Today*, 2008, **3**, 40–47.
18. S. Yean, H. J. Shipley, A. T. Kan and M. B. Tomson, Arsenate adsorption and desorption on magnetite nanoparticles: Effect of time and pH, 232nd ACS National Meeting, 2006, San Francisco.
19. D. Matschulat, H. Prestel, F. Haider, R. Niessner and D. Knopp, *J Immunol. Methods*, 2006, **310**, 159–170.
20. A. Baun, S. Sorensen, R. Rasmussen, N. Hartmann and C. Koch, *Aquatic Toxicol.*, 2008, **86**, 379–387.
21. G. Oberdörster, A. Maynard, K. Donaldson, V. Castranova, J. Fitzpatrick, K. Ausman, J. Carter, B. Karn, W. Kreyling, D. Lai, S. Olin, N. Monteiro-Riviere, D. Warheit and H. Yang, *Particle Fibre Toxicol.*, 2005, **2**, 8.
22. Draft report of FSA regulatory review, A review of potential implications of nanotechnologies for regulations and risk assessment in relation to food, March 2006, Food Standards Agency, London, www.food.gov.uk/multimedia/pdfs/nanotech.pdf.
23. Q. Chaudhry, M. Scotter, L. Castle, J. Blackburn, A. Boxall, R. Aitken and B. Ross, Assessment of the potential use of nanomaterials as food additives or food ingredients in relation to consumer safety and implication for regulatory controls, Final Report on Project A01057, 2007, Food Standards Agency, London.
24. Q. Chaudhry, L. Castle, E. Bradley, J. Blackburn, R. Aitken and A. Boxall, Assessment of current and projected applications of nanotechnology for food contact materials in relation to consumer safety and regulatory implications, Final Report on Project A03063, 2008, Food Standards Agency, London www.food.gov.uk/science/research/researchinfo/contaminantsresearch/contactmaterials/a03prog/a03projlist/a03063/.
25. M. R. Taylor, Assuring the safety of nanomaterials in food packaging: The regulatory process and key issues. Washington DC, Woodrow Wilson International Centre for Scholars, 2008, p. 6.
26. P. Šimon, Q. Chaudhry and D. Bakoš, *J. Food Nutr. Res.*, 2008, **47**, 105–113.

Subject Index